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Annales Medicinae Experimentalis et Biologiae Fenniae

Yrjönkatu 17, Helsinki, Finnland.

FROM THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY, UNIVERSITY OF HELSINKI

SOME FACTORS AFFECTING THE WASSERMANN REACTION

(ON FACTORS AFFECTING THE RESULTS OF THE WASSERMANN REACTION AND THE SIGNIFICANCE OF THE VARIATION IN RESULTS FROM THE STANDPOINT OF RELIABILITY OF SYPHILIS DIAGNOSIS)

By

KARI PENTTINEN and JUHA HELLE

(Received for publication 26. 7. 1948.)

This study attempts to elucidate to what extent the variation in the results obtained with the same specimen is due to the technical performance of the test, and to what extent this is caused by changes which have taken place in the serum. For this purpose several successive and parallel determinations were performed on specimens of serum. Special attention was paid to cases in which determinations made on two successive days gave a result that had changed from a clearly positive to a negative one. Such a change for the negative can have a great significance, since it appears that in some laboratories a simple qualitative test is followed on the next day by another test made in order to check the results of the first test and that the result yielded by the latter test is relied upon without paying attention to the result of the original test.

Method. — As to the method used at the University Department of Serology and Bacteriology we refer to previous reports (1, 2, 3). The result of the Wassermann test is considered here to have undergone a change if the result shows a change of two degrees

or more when the readings +, +?, ±, —?, — are applied. *In all test series, both ordinary Wassermann and chol. Wassermann changes were taken into consideration, all the tables thus showing the sum of the changes obtained in the same specimen, with both extracts. Therefore the number of changes would have been much smaller had only one Wassermann system been used.*

RESULTS

I. — Parallel determinations were made on the same Wassermann-positive serum, using both an automatically measuring pipette and the routine pipetting method. The total volume was 0.5 ml. (The use of small total volumes was imposed by the large size of the series and the small amounts of complement available). The result is illustrated in Table 1.

TABLE 1

COMPARISON BETWEEN AUTOMATIC PIPETTE AND ORDINARY ROUTINE PIPETTING METHOD. TOTAL VOLUME 0.5 ML

Result of Parallel Determinations								Specimens Examined
Automatic Pipette				Ordinary Pipette				
Same		Divergent		Same		Divergent		
n	%	n	%	n	%	n	%	n
113	96	5	4	95	81	23	19	118

It is evident that the use of an automatic pipette greatly stabilises the results.

II. — Parallel determinations were made on the same Wassermann-positive serum, using the ordinary total volume of 0.5 ml in one and of 1.25 ml in the other. Table 2 illustrates the results.

Table 2 shows that by increasing the total volume from 0.5 ml to 1.25 ml a decrease in the number of divergent results by nearly one-half is achieved. It is noteworthy that the number of divergent results is practically the same in both series of a total volume of 0.5 ml carried out separately (Tables 1 and 2). Thus, in case an automatic pipette is not available, the results may be stabilised by increasing the total volume used in the reactions.

TABLE 2

COMPARISON BETWEEN THE TOTAL VOLUMES OF 0.5 AND 1.25 ML

Result of Parallel Determination								Specimens Examined
0.5 ml				1.25 ml				
Same		Divergent		Same		Divergent		
n	%	n	%	n	%	n	%	n
93	80	23	20	105	91	11	9	116

III. — In the above series the tubes for parallel determinations were placed side by side in the series, usually right at the beginning. In order to throw light on the question whether the position of the tubes in the series has any bearing on the results, one of the tubes for parallel determination was placed in this series at the beginning and one at the end. It may be mentioned that the extent of the series was usually about 200 specimens. The result is shown in Table 3.

TABLE 3

COMPARISON BETWEEN PARALLEL DETERMINATIONS AT BEGINNING AND END OF SERIES. TOTAL VOLUME 0.5 ML

Result of Parallel Determinations				Specimens Examined
Same		Divergent		
n	%	n	%	n
73	72	29	28	102

It seems as if the placing of the parallel determination tubes at the opposite ends of the series increases the divergency of results. Of the side-by-side parallel determinations carried out with the same specimens 19 per cent again showed diverging results, just as they did in the tests illustrated by Tables 1 and 2. It is noteworthy that when placing the tubes at the beginning of the series a less positive result was obtained in 22 cases out of 29 than when placing them at the end of the series, whereas only 7 specimens placed at the end gave a less positive result than the one obtained at the beginning. The fact that the tubes at the beginning of the series gave a less positive result may be due the fact that when the first reading,

the so-called ten-minute reading, is carried out, the tubes at the end of the series, according to our observations, come to be about two minutes less in the thermostat because the reading is done at a faster rate than the filling of the thermostat.

IV. — In the following series the determinations on the same specimens were made on successive days. A total of 590 Wassermann-positive sera were examined. The results are illustrated by Table 4.

TABLE 4

COMPARISON OF DETERMINATIONS MADE ON SUCCESSIVE DAYS. TOTAL VOLUME 0.5 ML

Result of Determinations on Successive Days				Specimens Examined
Same		Divergent		
n	%	n	%	n
360	61	230	39	590

Table 4 shows that the number of divergent results increases still more when compared to the results presented in Tables 1, 2, and 3, if the determinations on the same specimens are carried out on successive days. Of the 230 cases which yielded divergent results, 142 (62 per cent) were less distinctly positive on the following day and 88 (38 per cent) more distinctly positive. The predominance of less definite results is statistically convincing. This is consistent with SIEVERS's (2) observation according to which in examinations of 20 Wassermann-positive sera a fall of the titre was sometimes found on preservation of the serum.

A fully negative result on the following day was found with regard to 92 sera. These cases will be dealt with in detail later.

Special interest is aroused by the preponderance of the less distinct results obtained on the following day. Is there a decrease in the reagin amount already after a 24 hours-storage and after inactivation, or is the serum's capability to react otherwise weakened? Since, however, it was observed in series III that the position of the tube in the series may affect the result, we grouped the 590 sera examined according to what the position of the tube was on the first and second day of determination. Table 5 shows how the position of the tube in the series affected the results.

TABLE 5

VARIATION OF RESULTS WITH CONSIDERATION OF POSITION OF TUBE IN SERIES MADE ON TWO SUCCESSIVE DAYS

Variation	Group							
	I		II		III		IV	
	n	%	n	%	n	%	n	%
—	59	65	71	54	175	65	55	56
Weakened	13	14	38	29	64	24	27	27
Intensified	19	21	23	17	29	11	17	17
	91	100	132	100	268	100	99	100

GROUP I: FIRST EXAMINATION IN FIRST HALF OF SERIES AND SECOND EXAMINATION AT ITS END

GROUP II: FIRST EXAMINATION IN FIRST HALF OF SERIES AND SECOND AT BEGINNING OF SERIES

GROUP III: FIRST EXAMINATION AT SECOND HALF OF SERIES AND SECOND EXAMINATION AT ITS END

GROUP IV: FIRST EXAMINATION AT SECOND HALF OF SERIES AND SECOND EXAMINATION AT BEGINNING OF SERIES

THE LINE OF DEMARCATION BETWEEN FIRST AND SECOND HALF IS THE MIDMOST TUBE OF THE SERIES

The result shown by Table 5 is consistent with that of table 3 concerning the effect of the tube's position, in so far as a possible preponderance of intensification is to be seen only in the first group, where the first reading was done in the first half of the series and the second at the end of it. The circumstance that in Groups II and III, where the effect of tube's position in the series is the slightest possible, a preponderance of weakening results is clearly discernible, also speaks for the assumption that the reactivity of sera has in fact weakened even within this short period of time.

When determining whether the predominance of weakened results is indeed due to the diminished capability of the sera to react, it should also be taken into consideration that there were 750 positive and probably positive responses in the results obtained from both extracts. They could only weaken when undergoing changes. There were 313 negative and probably negative responses, which could only be intensified when changing. Thus it would be possible to explain the predominance of weakening. Still it must be

regarded that the fluctuating possibilities of positive sera belonging to the +, +? group did not have nearly the same possibilities of variation as the —, —? group sera, since the former may be strongly positive and not likely to be affected by technical errors to the same extent as the latter group which only contained borderline cases. With regard to the possibilities of the fluctuation the ± group, containing 117 sera, forms a homogeneous part of the series. 33 of them remained unchanged, 47 were weakened and 37 intensified. This also suggests that the weakening may be due to a decrease in the reactivity of the serum.

Thus it may be possible that, irrespective of the technique, the tendency of the sera to weaken can even within the period of 24 hours cause a change in the Wassermann response and even render it negative. Since a change of this kind may easily be interpreted as a sign of false positivity, we have, with a view to the reliability of a syphilis diagnosis, directed our attention to those cases where a renewed examination gave a fully negative result. As already mentioned, the number such sera was 92. They had been taken from 84 different people. Table 6 presents these cases as grouped according to the probability of syphilis.

TABLE 6

84 CASES WHERE RENEWED EXAMINATION ON THE FOLLOWING DAY GAVE A NEGATIVE RESULT

Syphilis		Persistently Sero-positive	Insufficiently Studied	False Positivity
Certain	Probable			
52	11	9	9	3

Among the certain and probable cases there were 26 cases of neurosyphilis, 2 of cardiovascular syphilis, 22 of benignant late syphilis, 4 of congenital syphilis, 9 of primary and secondary syphilis. Attention is called to the high incidence of the diagnostically important neuro-syphilis. Table 6 makes it evident that a change of the result of the reaction to a fully negative one on the following day can by no means be regarded as a mark of unreliability of the previous day's result.

SUMMARY

1. Routine pipetting without the use of an automatic pipette providing accurate measurement is, to a considerable extent, liable to errors when a small total volume is used, which is evidenced above all by the variation in the result. Yet an exceedingly small number of results with false positivity is found.

2. It seems as if the reactivity of a positive serum could be weakened by storage for 24 hours and after-inactivation sufficiently to affect the results.

3. The change of a positive serum to a negative one in a re-examination carried out on the following day should not be considered a sign of false positivity.

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FROM THE DEPARTMENT OF PHYSIOLOGY, VETERINARY COLLEGE, HELSINKI

EFFECT OF HEAT ON SPERMATOGENESIS

PRELIMINARY COMMUNICATION

By

GUSTAF ELFVING and OSMO TURPEINEN

(Received for publication 7. 9. 48.)

It is a well established fact that the optimum temperature for mammalian spermatogenesis is a few degrees lower than the general body temperature and that the main physiologic function of the scrotum is to act as a relatively cool repository for the testes (1, 5). It is also known from numerous animal experiments (3, 4, 6) and from observations of the cases of human cryptorchidism (1) that spermatogenic tissue is remarkably sensitive to even slight elevations of temperature, so that an increase to the level of the general body temperature causes a cessation of spermatogenesis. While all this has been fairly extensively studied, there seem to be very few data (7) on the effect of short exposures to somewhat higher temperatures and, on the whole, the temperature — time relationships in the causation of testicular injury have received little attention. This problem was deemed interesting and important enough to merit some further study, of which the following is a preliminary communication.

Methods. — Male rats of proven fertility were used as experimental animals. They were placed upright in a narrow cylindrical cage and subjected to heat treatment in a water thermostat (Fig. 1), so that only the tail end of the body including the scrotum was submerged. The temperatures ranged from 41° C to 47° C and the times of exposure from 8 to 60 minutes. After this treatment the fertility of the male was tested from time to time by coupling the

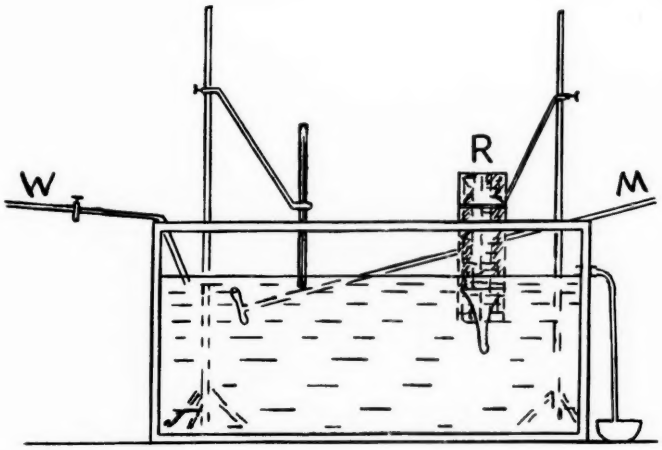

Fig. 1. — Water thermostat used in experiments.
W — hot water inlet, R — rat cage, M — mixer.

animal with an estrous female. The vaginal plug was searched for, and the presence and approximate density of sperm were noted. The female was isolated and observed for signs of pregnancy. If a litter was produced, its size and condition were noted.

Results. — The heat treatments had no appreciable effect on the readiness of the male to copulate or on the formation of the vaginal plug. They did have a marked influence on spermatogenesis and thus on the fertilizing capacity of the male. Sperm density in the plug decreased and soon all sperm disappeared. However, the male continued to copulate and produced sperm-free plugs for varying periods of time. Aside from this »organic» sterility, we observed occasional cases in which the sperm density was nearly normal but the copulations proved sterile. This »functional» sterility was mostly seen as a transitional stage between fertility and »organic» sterility.

The results obtained so far are shown in the Fig. 2. They may be summarized as follows.

41° — This temperature on exposures for 60 minutes had no distinct effect on fertility.

43° — Exposures for 20 minutes had only an uncertain effect, but 30-minute exposures caused sterility lasting several weeks.

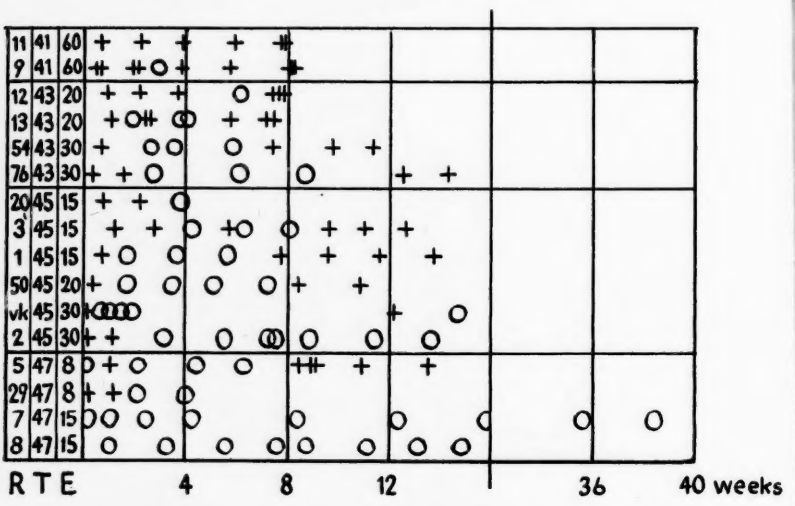

Fig. 2. — Fertility of male rats after heat treatment. Abscissa: time in weeks. R — rat number; T — temperature, °C; E — time of exposure, minutes; + — fertile copulation; O — sterile copulation.

45° — Exposures for 15 to 20 minutes were enough to cause cessation of spermatogenesis for several weeks.

47° — Relatively short exposures (8 minutes) stopped spermatogenesis for considerable periods of time. Exposures for 15 minutes caused a very long-lasting — possibly permanent — sterility.

It is evident from the data given in Fig. 2. that the males generally do not lose their fertilizing capacity immediately after the heat treatment. This phenomenon has also been noted by previous workers (7) and been shown to be due to the fact that the mature sperm stored in the epididymis is more heat resistant than the spermatogenic tissue itself and will enable the animal to remain fertile so long as these stores last, i.e., for some two to three weeks. Only a relatively rigorous heat treatment will also inactivate the epididymal spermatozoa and cause immediate sterility (cf. experiments at 47°).

The above studies are being continued.

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FROM THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY, UNIVERSITY OF HELSINKI

STUDIES ON THE OCCURRENCE OF HEMAGGLUTININS IN SEEDS

By

J. MUNTER

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LANDSTEINER and RAUBITSCHEK found (3) hemagglutinins from some species of the family of *Leguminosae* agglutinating red cells of different animals and human beings. Some extracts were found to agglutinate selectively the red cells of different species. They did not study the inward differences of the species. — EISLER and PORTHEIM (1), studying 99 species from 56 different genera, found hemagglutinins of this kind only from six species which all belonged to the genus of *Datura*. — SIEVERS (5) studied the effect of extracts of pea and bean upon the red cells belonging to the different blood groups, ascertaining that the different red cells are agglutinated roughly in the same way. — RENKONEN (4) studied 99 species from the family of *Leguminosae* and found a strong anti-A_1 and some anti-O (and anti-A_2) agglutinins. — With this as a starting-point, an attempt has been made by the present writer to find selective agglutinins from the other families.

Technique. — Seeds were cleaned from scales as well as possible and ground by hand in a mortar. The powder is extracted with saline (1: 9) for two hours at 37°C. After centrifugation, the clear solution was used as the primary solution (1: 1) to tests. 0.1 ml of this extract and 0.1 ml of 2 per cent fresh blood cell suspension from whole blood were pipetted into test tubes and kept at room

[1] The seeds used in the examinations were from the collections of the Botanical Garden of the University of Helsinki. The names are according to NILS HYLANDER: *Kärlväxter* (2).

temperature for one hour. The results were read by the naked eye either by shaking slightly the test tube, or, in order to see more sensitive positive results, by inclining the rack from the bottom of the tube.

All tests were performed chiefly on the blood cells from same persons belonging to the blood groups A, B and O. According to the results the series was grouped into 1) families containing only non-agglutinating and 2) families containing, also agglutinating species.

1. FAMILIES CONTAINING ONLY NON-AGGLUTINATING SPECIES

Lycopodiaceae:
- Lycopodium annotinum
- » complanatum

Taxaceae:
- Taxus baccata

Cupressaceae:
- Juniperus communis

Pinaceae:
- Pinus silvestris
- Picea Abies

Sparganiaceae:
- Sparganium minimum
- » ramosum

Potamogetonaceae:
- Potamogeton natans
- » praelongus

Scheuchzeriaceae:
- Scheuchzeria palustris

Alismataceae:
- Alisma Plantago-aquatica

Graminae:
- Anthoxanthum odoratum
- Milium effusum
- Alopecurus pratensis
- » arundinaceus
- Agrostis stolonifera
- Deschampsia caespistosa
- » bottnica
- » flexuosa
- Avena sativa
- Cynosurus cristatus
- Secale cereale
- Elymus arenarius
- Hordeum distichum

Cyperaceae:		
	Scirpus silvaticus	
	» Tabernaemontani	
	» rufus	
	Carex dioeca	
	» pulicaris	
Araceae:		
	Calla palustris	
Juncaceae:		
	Juncus conglomeratus	
	Luzula pilosa	
	» multiflora	
Liliaceae:		
	Paris quadrifolia	(hemol.)
Orchidaceae:		
	Orchis sambucina	
Corylaceae:		
	Corylus Avellana	
Betulaceae:		
	Betula pubescens	
	» nana	
	Alnus glutinosa	
	» incana	
Fagaceae:		
	Fagus silvatica	
Cannabaceae:		
	Cannabis sativa	
Urticaceae:		
	Urtica urens	
	» dioeca	
Chenopodiaceae:		
	Chenopodium album	(hemol.)
	Atriplex patula	(»)
	Bassia longifolia	(»)
Caryophyllaceae:		
	Stellaria media	
	» calycantha	
	Cerastium alpinum	
	Minuartia peploides	(hemol.)
	Moehringia trinervia	(»)
	Spergula arvensis	(»)
	» maxima	(»)
	» vernalis	(»)
	Agrostemma githago	(»)
	Lychnis Flos-cuculi	(»)
	Viscaria vulgaris	(»)
	» alpina	(»)

	Silene Cucubalus	(hemol.)
	Melandrium noctiflorum	(»)
	» rubrum	(»)
	Dianthus superbus	(»)
Ranunculaceae:		
	Actaea spicata	
	Caltha palustris	(hemol.)
	Aconitum septentrionale	
	Anemone Hepatica	
	» nemorosa	
	Ranunculus sceleratus	
	» auricomus	
	» acris	
	Myosurus minimus	
Papaveraceae:		
	Chelidonium majus	
	Papaver somniferum	
	Corydalis solida	
	» nobilis	
Cruciferae:		
	Brassica oleracea	
	» Napus	
	Sinapis alba	
	Cakile maritima	
	Lepidium ruderale	
	Isatis tinctoria	
	Thlaspi arvense	
	Capsella Bursa-pastoris	
	Cochlearia danica	
	Arabidopsis Thaliana	
	Turritis glabra	
	Erysimum Hieraciifolium	
	» cheiranthoides	
	Sisymbrium officinale	
	Camelina Alyssum	
	Descurainia Sophia	
Geraniaceae:		
	Geranium silvaticum	
	» pratense	
	» bohemicum	
	» Robertianum	
	Erodium cicutarium	
Linaceae:		
	Linum usitatissimum	
Rhamnaceae:		
	Rhamnus catharticus	
	» Frangula	

Tiliaceae:		
	Tilia cordata	
Guttiferae:	Hypericum maculatum	
Cistaceae:		
	Helianthemum nummularium	
Droseraceae:		
	Drosera rotundifolia	
Violaceae:		
	Viola collina	
Thymeleaceae:		
	Daphne Mezereum	
Elaegnaceae:		
	Hippophaë Rhamnoides	
Onagraceae:		
	Epilobium collinum	
	» roseum	
Umbelliferae:		
	Anthriscus silvestris	
	Myrrhis odorata	
	Conium maculatum	
	Cicuta virosa	
	Carum Carvi	
	Aegopodium podagraria	(hemol.)
	Aethusa Cynapium	
	Angelica Archangelica ssp. litoralis	
	Heracle m spondylium ssp. sibiricum	
Ericaceae:		
	Pyrola rotundifolia	
	Chamaedaphne calyculata	
	Arctostaphylos alpina	
	Vaccinium Myrtillus	
	» Vitis-idaea	
Empetraceae:		
	Empetrum nigrum	
Primulaceae:		
	Primula veris	
	» farinosa	
	Lysimachia vulgaris	(doubtful)
	Trientalis europaea	
Menyanthaceae:		
	Menyanthes trifoliata	(hemol.)
Oleaceae:		
	Fraxinus excelsior	
Convolvulaceae:		
	Convolvulus arvensis	(hemol.)
Polemoniaceae:		
	Polemonium acutiflorum	(hemol.)

Boraginaceae:

Lithospermum arvense
Echium vulgare
Lappula Myosotis
Cynoglossum officinale (hemol.)

Labiatae:

Galeopsis Tetrahit
» speciosa
Origanum vulgare
Lycopus europaeus
Mentha arvensis

Scrophulariaceae

Scrophularia nodosa
Castilleja pallida
Melampyrum nemorosum
Euphrasia frigida
Odontites rubra
Bartsia alpina

Plantaginaceae:

Plantago major
» maritima

Rubiaceae:

Galium verum
» Mollugo

Adoxaceae:

Adoxa Moschatellina

Caprifoliaceae:

Lonicera Xylosteum

Campanulaceae:

Campanula rapunculoides
» latifolia

Compositae:

Anthemis arvensis
Achillea Millefolium
Chrysanthemum vulgare
» Leucanthemum
» arcticum
Matricaria discoidea
Artemisia vulgaris
Senecio Jacobaea
Arctium minus
» tomentosum
Carduus nutans
Cirsium vulgare
Centaurea Jacea
» Cyanus
Lapsana communis
Taraxacum erythrospermum

2. FAMILIES CONTAINING BOTH AGGLUTINATING AND NON-AGGLUTINATING SPECIES

		Agglutination:
Polygonaceae:		
	Rumex crispus	weak
	» obtusifolius	»
	» Acetosella (coll.)	doubtful
	Polygonum aviculare	negative
	» Hydropiper	doubtful
Nymphaeaceae:		
	Nymphaea candida	negative
	Nuphar luteum	very weak
Crassulaceae:		
	Sedum Rosea	very weak
	» Telephium	» »
Saxifragaceae:		
	Saxifraga stellaris	negative
	» nivalis	»
	Ribes alpinum	very weak
Rosaceae:		
	Filipendula Ulmaria	weak
	» vulgaris	very weak
	Prunus Padus	negative
	Rubus Chamaemorus	doubtful
	» arcticus	weak
	» saxatilis	»
	Fragaria vesca	negative
	Potentilla strigosa	weak
	» argentea (coll.)	»
	» norvegica	»
	» Goldbachii	»
	» Crantzii	»
	» reptans	»
	Rosa glauca	negative
	» dumalis	»
	Sanguisorba minor ssp. dictyocarpa	»
	Sorbus intermedia	doubtful
	» aucuparia	negative
	Cotoneaster intergerrimus	»
	» melanocarpus	»
	Geum urbanum	weak
	» rivale	»
	» rivale x urbanum	»
Lythraceae:		
	Lythrum salicaria	weak

Solanaceae:

Atropa Bella-donna	negative
Hyoscyamus niger	»
» albus	»
Solanum Dulcamara	»
Datura Stramonium	strong
» ferox	»
» fastuosa	»

In all, 215 different species of seeds from 59 different families were examined. Altogether 192 species inactive were, from these 172 in 52 families with only non-agglutinating extracts, while 20 belonged to 7 families containing 23 agglutinating extracts, too.

Compared with the hemagglutinins in the family of *Leguminosae* and in the species of *Datura*, positive results in other families were very weak, less than 1/10, and could be ascertained mainly by means of the more sensitive method of estimation. Most of these agglutinins are found in the families of *Rosaceae*, *Saxifragaceae* and *Crassulaceae*, which all belong to the order of *Rosales*, to which the family of *Leguminosae* also belongs. Agglutinins are also found in the families of *Polygonaceae*, *Nymphaeaceae*, *Lythraceae* and *Solanaceae*.

No clear selectivity with regard to different blood groups could been found by this method among the weakly agglutinating extracts, nor in any of the more strongly agglutinating (1/80) species of *Datura*.

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THE SUBGROUPS A_1, A_2 AND A_1B, A_2B AND THEIR RELATION TO SOME HEMAGGLUTININS PRESENT IN SEEDS OF VICIA CRACCA

By

ROLF KOULUMIES

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K. O. RENKONEN (1948) observed that there are seed extracts containing fairly strong anti-A_1 and others containing anti-O (and anti-A_2) agglutinins. He observed that in titration of the *Vicia Cracca* extract the A_1 and the A_1B cells are more strongly agglutinated than others. The A_2 seem to be more sensitive than the B, O, or A_2B cells. The present investigation is made in order to ascertain whether the phenomenon is of value in a differentiation of the bloodgroups A_1, A_2, A_1B and A_2B. For this purpose blood specimens were taken from 100 A and 100 AB persons, a subgroup diagnosis was made, and they were titrated with the Vicia cracca extract.

Material and Technique. — The blood cells originated from the donors of the local Blood Service Centre.

The differentiating of subgroups was made in the following way: the so-called »absorbed B serum» (the anti-A_1 agglutinins), was prepared according to WIENER (1946) by absorption of the serum from a selected B individual with A_2 cells. Two bovine sera containing anti-A_2 agglutinins were absorbed with A_1B P cells after FRIEDENREICH and ZACHO (1931). — Since the anti-O agglutinins are unsuitable for differentiation of the AB group, the so-called absorption test was used in addition: B serum was absorbed by ¼ volume of the cells under examination and titrated thereafter with A_1 cells. Doubtful cases were further supplemented by making

an absorption curve, which was obtained when absorbing B serum by three different volumes (1/4, 1/16 and 1/64) of cells to be examined (DAHR 1943).

Vicia Cracca extract is made as follows: Seeds are powdered by hand in a mortar. The powder (1 part) is extracted with saline (99 parts) at 37° C for two hours. After centrifugation the solution

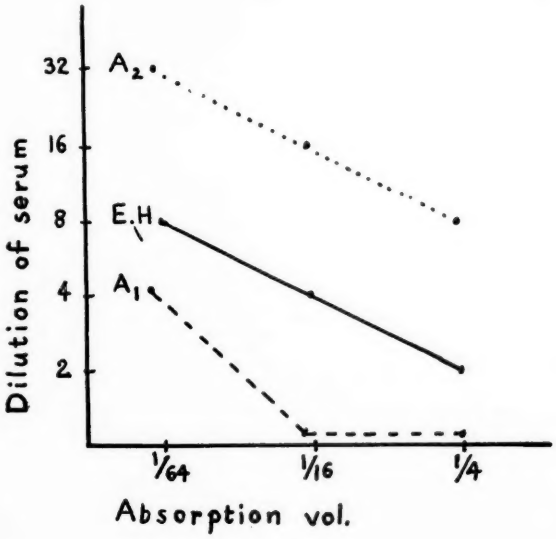

Fig. 1.

is used for the agglutination test. For the titration the extract is diluted with saline. The red cells were used fresh as a 2 per cent suspension of defibrinated blood in saline. 0.1 ml of the extract dilution and 0.1 ml of the red cell suspension are then pipetted into test tubes and kept at room temperature for one hour. The results are read by the naked eye.

Resulls. — Subgroups: Dividing group A into subgroups, there were 79 certain A_1- and 19 A_2-cells. 2 specimens (cases E. H. and O. S.) reacted both to anti-A_1- and anti-A_2-sera and were most consistent with those A cells which LANDSTEINER and LEVINE (1930) termed »intermediates».

Case E. H. reacted more weakly with A_1 (+) than with A_2 (++). The adsorption curve falls between the A_1 and A_2 curves used as controls. Fig. 1.

Case O. S. also reacted weakly with A_1 (+) and strongly with A_2 (+++). The absorption curve is in this case consistent with the A_2 used as control. Fig. 2.

The AB group was divided into 65 cases of A_1B and 35 of A_2B. No group of the »intermediate» type was found among the bloods examined.

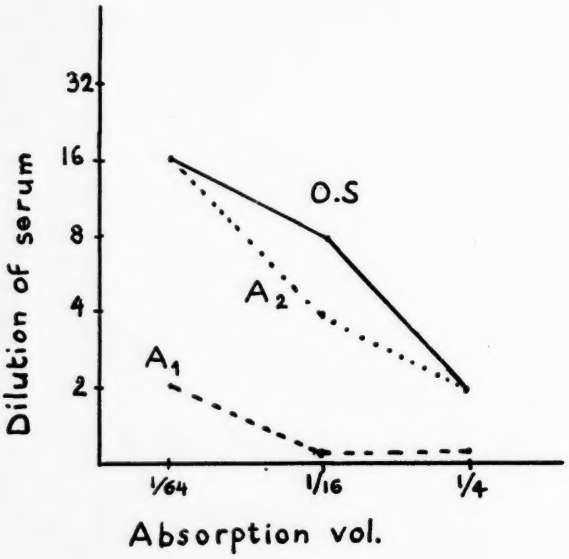

Fig. 2.

Vicia Cracca Titration. — The results of the Vicia titration are illustrated by the table. Four different degrees were used for the interpretation of positive agglutination (+++, ++, + and ±). The titre was the greatest dilution of the *Vicia Cracca* extract which still showed a strong reaction (+++ or ++). A weak reaction (+ or ±) was often still perceptible in the following dilution.

As the table reveals, all A_1 titres, with one exception (case T. A), lay between 1: 1600 and 1: 6400, with no case of A_2. Case T. A., whose titre was 1: 800, was proved A_1 in all tests, also in renewed ones, in which he gave the same reactions.

In the A_2 group half of the cases had a titre below 1: 100. One of them was fully negative (—) in a 1: 100 dilution and 9 had but a weak reaction (±) in this dilution. Neither did the other A_2,

TABLE 1

TITRATION OF VICIA CRACCA EXTRACT

Extract Dilution	Number of Bloods in Eeach Group				
	A_1	A_2	»Interme-diates» A	A_1B	A_2B
Below 1: 100		10	1 (O. S.)		28
1: 100		3			6
1: 200		4			1
1: 400		2			
1: 800	1 (T. A.)		1 (E. H.)	4	
1: 1600	25			40	
1: 3200	41			19	
1: 6400	12			2	
Totals	79	19	2	65	35

although their titre varied from 1: 200 to 1: 400, produce the strongest reaction (+++) in any tube studied, the maximum strength of reaction being in all of them ++.

Of the »intermediates» one (O. S.) reacted like A_2, the titre being 1: 100. The other (E. H.) also showed an A_2-like reaction, with Vicia extract, since in all dilutions examined the strongest intensity of reaction was only ++, notwithstanding the high titre of 1: 800.

The titres of the A_1B group were somewhat lower than those for A_1, the majority of them, *i.e.*, 40, having a titre of 1: 1600. Yet the intensity of the reaction was the same as for A_1 (+++).

The titres of group A_2B were also weaker than for A_2. In 17 cases no reactions manifested themselves in a 1: 100 dilution (—).

To summarise, it can be said that the investigation revealed the saline extract from seeds of *Vicia Cracca* to be equally suitable for a differentiation of subgroups A_1, A_2 and A_1B, A_2B as the absorpted anti-A_1 sera. A certain advantage of the former is the circumstance that it is easy to prepare and stabile.

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LIVER FUNCTION TESTS IN ACRODERMATITIS CHRONICA ATROPHICANS HERXHEIMERI

By

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Since it had been found that some liver function tests were positive in one case of acrodermatitis chronica atrophicans Herxheimeri treated at the Third Medical Clinic of the University of Helsinki, the writers have studied these tests in ten cases of this disease. In addition, the sedimentation rate of the blood and the diastase content of the urine were taken into account.

CASE MATERIAL

The patients were 9 women and one man (Table 1). Three patients were examined at the hospital, the rest at the out-patients' department. One (No. 5) had in addition a high blood pressure and congestive heart failure, two (No. 3, 10) extensive varicose veins in both legs. The youngest patient (No. 6) had a complication of essential hyperchromic anemia, with which she had been afflicted for a number of years. Two (No. 4, 8) had recently been treated with 1—3 million units of penicillin because of exanthema, one (No. 6) had been administered 1 million units because of puerperal inflammation of the uterine appendages. In one patient (No. 4) the bluish tint of the rash was unmistakably intensified in con-

TABLE 1

Case No.	Sex	Age	Onset of Exanthema	Region of Manifestation [1] of Exanthema
1	female	43	ca. 3 years ago	left hand and elbow +++; left leg +; right elbow +
2	»	64	ca. 6 » »	both legs, feet, and lower third of thighs +++; both elbows +
3	»	58	ca. 7 » »	both legs, feet, and lower third of thighs +++
4	»	38	ca. 10 » »	from ankles to halfway up the thighs on both lower limbs +++; left elbow +
5	»	57	ca. 12 » »	from left foot up to knee +++; right foot +
6	»	27	ca. 12 » »	right ankle, leg, and thigh +++; left leg and thigh ++ gluteal and lumbar regions +++; right elbow +++
7	»	50	ca. 15 » »	both lower extremities +++
8	male	41	ca. 20 » »	both elbows +++; both hands and wrists +++; both feet, legs, knees +++
9	female	64	ca. 23 » »	right foot +++; left ++; left elbow +++; both knees +
10	»	64	ca. 25 » »	both lower extremities ++

[1] +++ = severe skin changes
++ = moderate
+ = slight.

nection with menstruation. Periodical pains in different joints manifested themselves in three patients (No. 1, 4, 5). One patient (No. 2) suffered from hyperchromic tapeworm anemia.

METHODS

The specimens were taken in the morning on fasting. The blood samples were examined fresh. The diastatic content of the urine was determined from the morning urine according to Wohlgemuth.

TABLE 2

Case No.	SR mm/1 h.	Takata	Stolte	Thymol Turbidity Units	Seiffert-Nagler Modification
1	10	negat.	2.00 ml.	3	+2
2	34	negat.	1.20 ml.	15	+1
3	31	negat.	1.42 ml.	12	+1
4	44	+	1.38 ml.	16	+2
5	98	++	1.22 ml.	19	±0
6	26	negat.	1.76 ml.	10	+1
7	40	++	1.24 ml.	15	+2
8	32	+(?)	1.54 ml.	10	+2
9	35	negat.	1.34 ml.	9	+2
10	78	negat.	1.42 ml.	8	+2

For the liver function tests, the TAKATA-ARA test was used as well as its modification after Stolte's titration method, Maclagan's thymol turbidity test, and modification of the serum test based on Seiffert-Nagler's phenomenon.

RESULTS

The sedimentation rate (Table 2) was within the normal limits in one patient only (No. 1). Her exanthema was the least serious and had begun later than that of the other patients. (Table 1). One patient (No. 4) came for hospital treatment because of an increased sedimentation rate (over 50 mm/1 h.) during several years without any known reason. After penicillin treatment (in all 3 million units) her sedimentation rate was reduced to 44 mm/1 h., the strongly positive Takata-Ara test became weakly positive, and the positive Stolte test (1.20 ml) negative (1.38 ml.). In case No. 8 the penicillin treatment (1 mill. units) did not affect the sedimentation rate. With the exception of the exanthema, nothing of any significance was found in this patient.

The Takata-Ara test (Table 2) was clearly positive in two cases only (No. 5, 7). One of them (No. 5) revealed, in addition to increased blood pressure, congestive heart failure. The Stolte test was found to be positive in one patient (No. 2). In case No. 6 where the exanthema had the widest distribution, both the Takata-Ara and the Stolte tests were negative.

The thymol turbidity test was within normal limits in one patient only (No. 1). In the nine other patients examined the test was clearly positive.

The serum test, based on the Seiffert-Nagler phenomenon (1, 2) was within normal limits in all the ten cases. (This test followed closely the thymol turbidity test in over 500 cases examined on a different occasion, being, however, more sensitive for hepatitis

TABLE 3

Case No.	Diastase Content of Urine
1	$2^7 = 128$ WE [1]
2	$2^7 = 128$ WE
3	$2^4 = 16$ WE
4	$2^7 = 128$ WE
5	$2^4 = 16$ WE
6	$2^5 = 32$ WE
7	$2^5 = 32$ WE
8	$2^6 = 64$ WE
9	$2^6 = 64$ WE
10	$2^6 = 64$ WE

[1] Wohlgemuth units.

serum. The normal values were + 1 and + 2, the values for hepatitis serum from + 3 to + 10. The only cases where this test was negative, in spite of a clearly positive thymol turbidity test, were those of acrodermatitis, which speaks for the assumption that serum alterations in this desease are not similar to those of parenchymatous hepatitis.)

The diastase content of the urine (Table 3) had risen to the threshold-value of 128 Wohlgemuth units in three cases (No. 1, 2, 4). In these instances the skin changes had set in during the past ten years.

SUMMARY

1. Ten cases of acrodermatitis chronica atrophicans Herxheimeri have been examined.

2. The Takata-Ara test was clearly positive in two cases.

3. The Stolte test was positive in one case.

4. The thymol turbidity test was positive in 9 cases.

5. The serum test based on the Seiffert-Nagler phenomenon was negative in all cases.

6. The sedimentation rate was increased as a rule.

7. The diastase content of the urine had increased in three cases to the threshold-value.

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COMPARISON OF PERORAL FRUCTOSE AND SUCROSE TOLERANCE TESTS IN NORMAL RABBITS

By

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The normal liver has the ability to eliminate almost all the fructose carried to it by the portal blood. In a diseased liver, however, a greater or smaller portion of the absorbed fructose escapes into the general circulation, and fructose can be detected both in peripheral blood and in urine. Several modifications of the fructose tolerance test have been used as a help in the diagnosis of hepatic disease. Either blood fructose (10, 24, 8) or the urinary excretion (25, 22, 3, 11) have been analysed. In addition, a third type of test has been based on the observation that fructose does not normally give rise to as high an increase in the blood sugar as a corresponding amount of glucose (20, 22, 2, 3, 9, 24).

During the last few years, fructose tolerance tests have perhaps not been used to the same extent as some other chemical methods of studying the hepatic functions. It was, however, thought that a re-investigation of the subject from a new angle would be justified.

The use of sucrose instead of fructose may be expected to offer some advantages. Ingested sucrose is hydrolysed — by HCl in the stomach and by invertase in the intestine — to its components, fructose and glucose; 100 g of sucrose yields 52.6 g of each sugar. Sucrose is easily available and cheap in comparison to fructose, the use of which may in some circumstances be limited also because of economical considerations. Recent improvements in the fructose

determination method (4) have made the estimation of fructose a simple task which can be rapidly performed and gives satisfactory results without the need of training long the analyst. Previously the effect of sucrose on the total sugar value of blood has been investigated in rabbit by REINHOLD & KARR (18) and the effect on both blood fructose and total sugar levels by OPPEL (15). The present writers, however, have failed to find in the literature any systematic comparison of sucrose and fructose tolerance tests.

The purpose of the present work was to ascertain whether great doses of sucrose give similar blood fructose concentrations as fructose in approximately equivalent amounts (i.e., half the weight of a dose of sucrose). Total blood sugar values were also determined.

MATERIAL AND METHODS

Altogether 65 sucrose and fructose tolerance tests were performed on six adult rabbits, weighing from 1½ to 2½ kg. The weight of the rabbits fluctuated during the experimental period at a maximum 360 g. The dose of sugar was dissolved in 50 cc of water, and the solution was administered with a stomach tube. Blood samples, of 0.4 cc each, were taken from the ear veins. In most experiments, the procedure was as follows: (1) a fasting value, (2) administration of sugar, thereafter (3—7) samples 30, 60, 90, 120 and 180 minutes after the administration of sugar. In a few tests, the blood sugar was followed only for 120 minutes, and the intervals of the samples were 20 minutes.

Protein Precipitation. — Duplicate samples of blood, of 0.2 cc each, were pipetted into 2.0 cc of distilled water. After the haemolysis was complete, approximately 10 mg of solid sodium fluoride was added to the solution, in order to prevent glycolysis. The proteins — and the non-sugar reducing agents — were removed with a cadmium hydroxide precipitation, according to the method of FUJITA & IWATAKE (7). 1.6 cc of the cadmium reagent (13.0 g of $CdSO_4$ and 63.5 cc of 1 N H_2SO_4 in 1 litre of water) and 0.2 cc of 1.1 N NaOH were added to the haemolysate. The tubes were stirred and centrifuged. 0.5 cc of the clear supernatant fluid was used for the fructose determinations and 2.5 cc for the total sugar.

Fructose Determination. — The fructose determinations were performed according to COLE's modification of ROE's (19) method. The reagents needed are a 0.15 per cent solution of resorcinol in absolute ethanol and a 0.75 mg per cent $FeCl_3$ solution in concentrated HCl. 0.5 cc of the protein-free fluid, 1.5 cc of distilled water, and 3.0 cc of each reagent were incubated at 75° C for 30 min., in wide test tubes with loosely fitting bulb-formed glass stoppers. The tubes were cooled with running water, and the intensity of the resulting red colour was measured with a Pulfrich photometer (filter S 50, optic depth 20 mm). A blank determination was used on the com-

parison side of the photometer. As colour standards a 5 mg per cent and a 1 mg per cent fructose solution were made daily and prepared parallelly with the unknown samples. The accuracy of the method on these dilute solutions was ± 10 per cent.

Total Reducing Sugar. — 2.0 cc of the protein free fluid was mixed with 2.0 cc of SOMOGYI's (21) copper reagent. The samples were kept for 10 minutes in a boiling water bath, in similar tubes as in the fructose determinations. They were cooled at room temperature; then 0.2 cc of a 2.5 per cent alkaline KJ solution and 0.6 cc of 2 N H_2SO_4 were added. The samples were titrated with 0.005 N thiosulphate. The method was controlled daily by preparing a 100 mg per cent fructose or glucose standard solution and by analysing it; both sugars gave identical titration values. Strict adherence to the stereotypical performance of all the operations was essential for obtaining agreeing duplicate determinations. In the hands of our analyst, the maximum discrepancy between duplicate determinations in this micro-method was 17 mg per cent, but the average difference was of the order of 6 mg per cent.

Sucrose Determination. — In COLE's method, the fructose solution is incubated with acid. If any sucrose is present in the solution, it can be expected to hydrolyse, and its fructose component will contribute to the fructose value of the solution. The concentration of sucrose can be determined by measuring the reduction before and after an acid hydrolysis. As sucrose is non-reducing, the difference may be assumed to indicate the sucrose concentration. In the sucrose determinations the method of LAVIETES, BOURDILLON & KLINGHOFFER (13) was followed. They hydrolysed the sucrose by adding 1.20 cc of 0.1 N HCl to 20 cc of the protein free blood filtrate, and incubating the mixture for 2 hours at + 85 to + 100° C. In our experience, the hydrolysis as performed according to their technique proved incomplete. When a 100 mg per cent sucrose solution was hydrolysed, 26 per cent of it was inverted within 30 minutes, 48 per cent in 60 minutes, only 72 per cent in 120 minutes, and in 240 minutes the hydrolysis was complete (104 per cent of the theoretical yield). In order to obtain complete hydrolysis in a shorter time, it was performed by using 10 times stronger acid, i.e., 1.20 cc of 1 N HCl to 20 cc of the protein free filtrate. After hydrolysis and cooling, the solution was correspondingly neutralized by adding 1.20 cc of 1 N NaOH, and the volume was made to 25 cc by adding distilled water. For a 100 mg per cent sucrose solution approximately theoretical reduction values were obtained within 30 to 120 minutes: after 30 minutes the value was 106 per cent, after 60 minutes 102 per cent, and after 120 minutes 98 per cent of the theoretical value. It was therefore regarded as sufficient to hydrolyse the samples for 30 minutes. Instead of the micro-method, 5 cc samples of the protein-free filtrate were used in the hydrolysis experiments.

RESULTS

Blood Fructose. — Large doses of sugar were necessary for obtaining measurable concentrations of fructose in peripheral blood. With a dose of 3.0 g of fructose or 6.0 g of sucrose per kg of body weight or more the fructose level generally rose to measurable values. The maximum blood fructose value recorded was 11 mg per cent. When the dosage was doubled, no corresponding increase was observed in the blood fructose values. Towards the end of the test the fructose level generally had a tendency to fall back, but the zero level was not always attained by the end of three hours. The fructose level was evidently independent of the changes in the total reducing sugar values; the fructose rose even in such tests in which there was no concomitant rise of the total sugar value.

The maximum fructose values attained in 45 tests are compiled in Table I and an average is calculated for each dosage. Similarly, the average fructose level during the course of each test is shown in Table II. Evidently, no significant difference can be observed between the results of the fructose and the corresponding sucrose tolerance tests.

TABLE I

MAXIMUM BLOOD FRUCTOSE MG PER CENT AFTER THE PER OS ADMINISTRATION OF FRUCTOSE (F) OR SUCROSE (S)

Rabbit	Dosage of Sugar g per kg of Body Weight						
	S 3.0	F 3.0	S 6.0	F 4.5	S 9.0	F 6.0	S 12.0
A	1.0	7.8	4.0	7.3	8.1	2.7	1.8
	0.8	1.9	3.9	3.6	11.0		
B	0	2.0	1.0	0	6.4	(7.6 [1])	
	0.9			2.7	1.8		
D	0	6.9	3.9	3.0	3.6	7.2	4.5
	0	0.9	0	2.7	1.8		
E	0	0	3.1	6.2	4.5	5.5	2.7
	2.0	5.2	1.0	3.6	7.2		
Averages	0.6	3.5	2.4	3.6	5.6	5.1	3.0

[1] Not taken into the average because there is no corresponding sucrose experiment.

TABLE II

AVERAGE BLOOD FRUCTOSE MG PER CENT DURING 3 (2) HOURS AFTER THE PER OS ADMINISTRATION OF FRUCTOSE (F) OR SUCROSE (S)

Rabbit	Dosage of Sugar g per kg of Body Weight						
	S 3.0	F 3.0	S 6.0	F 4.5	S 9.0	F 6.0	S 12.0
A	0.4	6.2	2.1	5.3 [2]	4.3	0.8	1.7
	0.2	1.0	3.0	1.9	4.3		
B	0 [2]	0.7	0.8	0 [2]	4.0	(2.7 [1])	
	0.2			1.4	1.1		
D	0	4.5	3.2	2.6	1.8	2.3	3.1
	0	0.5	0	1.4	1.5		
E	0	0	1.9	2.5	4.2	3.3	2.0
	0.5	3.1	0.2	1.6	2.3		
Averages	0.2	2.3	1.6	2.1	2.9	2.1	2.3

[1] Not taken into the average, because there is no corresponding sucrose experiment.
[2] Samples were taken only during two hours.

Blood Sucrose. — There is a general agreement that in normal adult mammals ingested sucrose is quantitatively hydrolysed before adsorption (16). After the administration of large doses of sucrose to children, some of it, however, can be found in the urine (12). A sucrosuria may occasionally follow also the administration of fructose or glucose alone (1, 17), and therefore the presence of sucrose in urine may not — *per se* — necessarily indicate its presence in blood. In a few instances, the presence of sucrose in blood has, in fact, been reported (1). When once gained access to the blood, sucrose is not utilized at all, and it is excreted quantitatively through the kidneys (13).

A priori the possibility could not be entirely excluded that the fructose values obtained in the sucrose experiments would have been due to sucrose hydrolysed in the course of the fructose determinations. For the investigation of this possibility three blood sucrose determinations were performed on blood taken 60 minutes after the administration of 9.0 g of sucrose per kg of body weight. After the hydrolysis the reduction value was the same as before, within the limits of the experimental error. As the blood fructose levels simultaneously recorded were of the same order as the experi-

mental error of the total reducing sugar method, the presence of a slight sucrosaemia might have escaped attention because of methodical inaccuracy. There is, therefore, no conclusive direct evidence for or against a slight sucrosaemia.

As the methods were regarded as inadequate for obtaining accurate direct blood sucrose determinations, an *indirect approach* was made. In 12 tests, made on two rabbits, the effect of various doses of sucrose and that of chemically equivalent doses of a mixture of equal parts of glucose and fructose were compared. If a significant sucrosaemia would have occurred in the sucrose tests — and not in the other series — it was to be expected that the fructose values in those tests would have been consistently higher than after administering the mixture of the two monosaccharides. The apparent fructose level, would, of course, have received an additional contribution from the blood sucrose. The main results of these experiments are shown in Table III. It is evident that no systematic difference has prevailed between the two series of tests. It may, therefore, be concluded, that the blood fructose values after the administration of sucrose have not been due to a sucrosaemia any more than after giving the monosaccharides. On the basis of the present results, the possibility of a slight sucrosaemia, however, can in neither case be entirely excluded.

TABLE III

THE MAIN RESULTS OF EXPERIMENTS IN WHICH THE EFFECT OF SUCROSE (S) AND THAT OF A CHEMICALLY EQUIVALENT AMOUNT OF A MIXTURE CONTAINING EQUAL PARTS OF FRUCTOSE (F) AND GLUCOSE (G) WERE COMPARED

Rabbit	Dosage of Sugar g per kg of Body Weight					
	F+G 3.16+3.16	S 6.00	F+G 4.74+4.74	S 9.00	F+G 6.32+6.32	S 12.00
	Maximum Blood Fructose mg-%					
H	3.9	1.8	6.3	8.0	6.8	3.2
I	3.5	5.9	5.3	6.7	4.7	3.6
	Average Blood Fructose mg-% during 3 Hours					
H	2.5	0.7	3.5	6.4	3.9	1.6
I	1.8	3.2	1.7	4.0	4.1	2.5
	Maximum Rise of Total Sugar mg-%					
H	104	51	21	45	177	30
I	116	140	76	117	160	100

Total Blood Sugar. — At the beginning of the series of tests, the basal (pre-experimental) sugar values varied between 36 mg per cent and 224 mg per cent; generally they were between 100 and 170 mg per cent. When experiments were repeated at short intervals, the basal sugar fell fairly uniformly in all rabbits. Fig. 1 shows the

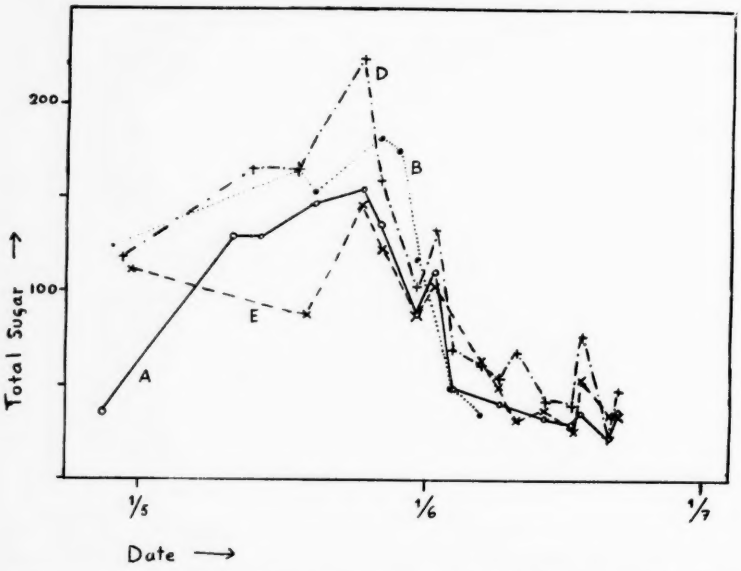

Fig. 1. — The basal blood sugar values of four rabbits throughout the experimental season.

fasting sugar values of four rabbits throughout the experimental period. The basal values were regularly below 80 mg per cent during the last month.

After the administration of the sugars there occurred a rise in the blood sugar in all but three tests. All the three tests without any rise were fructose tests performed on rabbit D. The rise was 177 mg per cent at a maximum. On an average the rise was higher in the sucrose tests than after giving fructose, but the dispersion of the individual tests is too high to give statistical significance to this — actually expected — difference. The data are collected in Table IV.

When the rise of the blood sugar was related to the fasting sugar value, no obvious relationship could be found. It seemed that the rise was independent of the previous sugar level. Neither was there

TABLE IV

MAXIMUM RISE OF TOTAL SUGAR MG PER CENT AFTER THE PER OS ADMINISTRATION OF FRUCTOSE (F) OR SUCROSE (S)

Rabbit	Dosage of Sugar g per kg of Body Weight						
	S 3.0	F 3.0	S 6.0	F 4.5	S 9.0	F 6.0	S 12.0
A	41	22	6	24	65	31	41
	29	44	57	62	46	24	41
B	90	20	52	23	39	(17 [1])	
	12			25	39		
D	16	—	70	—	30	37	23
	14	—	17	67	33	48	33
E	57	79	18	65	64	19	56
	17	34	18	76	50	33	11
Averages	35	28	34	43	46	32	34

[1] Not taken into the average, because there is no corresponding sucrose experiment.

any conclusive evidence for a systematic change in the rise with the proceeding of the experimental season. The only observation which points out to this direction is the relatively slight rise after the greatest doses of fructose and sucrose; they were administered at the end of the series. The slight rise, however, can equally well be ascribed to a longer stay of the highly concentrated solutions in the stomach (14).

DISCUSSION

When the present investigation was started, two possibilities were considered. First, that the fructose tolerance would be unaffected by the simultaneous administration of the same amount of glucose, as occurs when sucrose is given *per os*. The evidence presented by CORLEY (6) made this possibility seem likely. Second, that — in an analogy to the galactose tolerance test — the blood fructose level would depend on the simultaneous administration of glucose (5, 23). It turned out, that — as far as the present tests indicate — the fructose level of blood was the same whether the fructose was administered alone or in the form of sucrose. The possibility of slight differences can not be entirely excluded, but they are not manifest in experiments of this type.

The series of tests as a whole obviously affected the carbohydrate metabolism on the rabbits. Towards the end of the experimental period the rabbits showed a much lower basal blood sugar level than at the beginning. This was not due to too intense fasting, as the weight of the rabbits at the same time kept fairly steady. The fructose level evidently was unaffected by this change. Therefore, it may be concluded that the utilisation of fructose by the liver has not undergone any significant variations at the same time as the carbohydrate metabolism of the animal was trained by the tolerance tests.

The gradual decrease of the basal sugar values might also be explained by assuming that the animals had become used to the handling, and therefore they did not react any more with a release of adrenalin, when taken out of their cages for the experiments. This factor, however, can hardly have been the sole cause of the gradual fall, as the basal sugar values towards the end of the series were unusually low, of the order of 30 or 40 mg per cent. It must be kept in mind, of course, that SOMOGYI's method indicates true blood sugar values, whereas a considerable and varying proportion of the so-called blood sugar values — as determined with most of the other common methods — is made up by non-sugar reducing substances. Even so, the basal values are on the low side for animals in a good nutritional status.

The administration of strong sugar solutions *per os*, however, leaves one variable uncontrolled. The stomach tends to dilute hypertonic solutions before they are brought into the intestine. It has been shown that the stronger the sugar solution, the more slowly it is emptied from the stomach (14). In the present experiments it was necessary to limit the volume of the fluid to suit the capacity of the stomach. Therefore, the solutions administered were rather concentrated. The arrival of the sugar solution into the intestine was thus uncontrolled. That this variable factor has not seriously invalidated the results is shown by the fact that the fructose levels were not consistently different in the fructose and sucrose series.

The results of the present series of experiments evidently suggest that sucrose can be used instead of fructose in tolerance tests. This findings has to be, however, still confirmed on human patients.

SUMMARY

1. 65 tolerance tests were performed using the *per os* administration of sucrose, fructose, or a mixture of fructose and glucose. The total reducing blood sugar and blood fructose levels were followed.

2. Measurable blood fructose levels were regularly obtained after giving at least 6.0 g of sucrose per kg body weight or a corresponding amount of fructose — i.e., half the dose of sucrose. No systematic difference existed between the administration of fructose alone or as a component of sucrose.

3. The basal sugar values showed a considerable drop towards the end of the experimental season. The changes of the total blood sugar after administering either fructose or sucrose varied greatly.

4. There was no evidence for the absorption of sucrose as such.

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FROM THE DEPARTMENT OF MEDICAL CHEMISTRY, UNIVERSITY OF HELSINKI,
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A STUDY OF THE BLOOD PHOSPHORUS AND CERTAIN LIPIDS IN CHRONIC CARBON MONOXIDE POISONING

By

OSMO HELVE

(Received for publication 1. 12. 48)

During the war ending 1944 and for about three years after its close there was a lack of liquid fuel in Finland and, as a result, carbon monoxide generated from wood and charcoal had to be used in internal combustion engines. Carbon monoxide poisoning became common especially among people whose employment involved contact with motor cars, and often, notably in its chronic form, this occupational disease was difficult to diagnose. — The clinical signs of carbon monoxide poisoning are very diversified depending upon the intensity of the intoxication. The literature contains only a few publications (besides those on CO and hemoglobin) dealing with the metabolism in this intoxication. From the point of view of intermediary metabolism it seemed worth while to study the phosphorus and lipid metabolism in this connection.[1] No earlies studies of these metabolisms in cases of carbon monoxide poisoning have come to my notice.

MATERIAL AND METHODS

The series included 17 patients with carbon monoxide poisoning treated at the Clinic for Occupational Diseases of the University of Helsinki. All the patients were men, the great majority chauffeurs. Only clinically

[1] I wish to thank the Occupational Medicine Foundation for the grant which I received from this institution.

verifie l cases were selected, in which the CO content of the blood was also determined. The blood samples were extracted after the patients came under hospital observation and treatment. Because the blood phosphorus is chiefly derived from the red cells, attention was directed, besides to the volume of the red cells, also to their number and hemoglobin content. The number of leukocytes was studied because they also contain a considerable amount of phosphorus. All blood tests were taken in the morning after an overnight fast.

The *blood phosphorus* was determined as follows: The protein was removed from oxalated blood by trichloracetic acid and the phosphorus of the filtrate was then determined, in the main according to BOMSKOV's (1932) method. — The inorganic phosphate was determined according to FISKE and SUBBAROW (1925, 1929) and LEHMANN and JENDRASSIK (1926). — The pyrophosphate-P was obtained by hydrolysing trichloracetic acid filtrate of blood in 1 N hydrochloric acid at 100°C for 7 minutes. — The hexosephosphate-P was obtained by subtracting the 7-minute hydrolysis value from the P-value determined in the same way, except that the duration of hydrolysis was 180 minutes. — The diphosphoglyceric-acid-P was determined by deducting the 180 minute hydrolysis value from the total acid-soluble phosphorus. — The total acid-soluble phosphorus and the total phosphorus was determined by burning a suitable amount of trichloracetic acid filtrate of the whole blood, or whole blood with sulphuric acid and nitric acid, and neutralising it with ammonia prior to the phosphorus determination. — The plasma phosphorus determinations were carried out exactly as described above and simultaneously. — The corresponding phosphorus contents of the red cells were calculated on the basis of the hematocrit value. — A more detailed description of the above procedure is given in a previous communication dealing with the blood phosphorus distribution in certain diseases of the blood (HELVE, 1946).

Of the *plasma lipids*, the cholesterol, ester cholesterol, and lipid phosphorus were determined. These lipids were extracted into ether-alcohol. — From the extract the cholesterol was determined colorimetrically on the basis of Liebermann-Burchardt's colour reaction. The cholesterol esters were determined by the same reaction after free cholesterol had been precipitated with digitonin solution.

The phosphorus content of the plasma lipid extract was measured after burning the organic substances, as described above. The ether-soluble phosphorus was obtained by extracting the evaporation residue of the extract into ether, the procedure being then continued as above. The amount of phosphatides was obtained by multiplying the phosphorus of the extract by 25. A more detailed description of the methods of lipid determination is found in the work published by VESA and KALAJA in 1939.

TABLE I

TABLE I

BLOOD PHOSPHORUS (P IN MG%).

(B = WHOLE BLOOD; P = PLASMA; E = ERYTHROCYTES; HCR = ERYTHROCYTE VOLUME [%]; ER = ERYTHROCYTES, MILL./C.MM.; H = HEMOGLOBIN (SAHLI); L = LEUKOCYTES, IN C.MM.)

No.	Initials	Age	Date of Test (1946)	Severity of Carbon Monoxide Poisoning	Inorganic Phosphate			Pyrophos-phate-P			Hexosephos-phate-P			Diphospho-glyceric-acid-P			Total Acid-soluble-P			Total P			Hcr	Er	H	L
					B	P	E	B	P	E	B	P	E	B	P	E	B	P	E	B	P	E				
1	L. N.	40	10. 5.	++	3.14	3.28	2.93	3.14	0.28	7.43	4.42	0.80	10.93	17.81	0	44.68	28.57	3.64	65.97	36.00	11.71	72.44	40	5.04	93	6300
2	S. K.	48	17. 5.	++	2.79	3.00	2.50	2.91	0.21	6.64	4.30	0	10.24	18.29	0	43.55	28.29	3.21	62.92	36.00	11.43	69.93	42	5.42	95	9100
3	S. A.	39	15. 3.	++	2.64	2.79	2.38	2.64	0.14	6.90	4.72	0.14	12.52	16.57	0	44.78	26.57	3.07	66.58	37.14	11.43	80.92	37	4.42	80	8150
4	A. P.	37	14. 6.	++	2.64	2.86	2.25	3.06	0.14	8.25	4.01	0.07	11.02	13.97	0	38.81	23.68	3.07	60.32	32.57	11.54	69.96	36	4.47	84	5700
5	V. J.	39	8. 5.	++	2.79	3.21	2.01	3.63	0.21	9.98	3.72	0.07	10.49	15.00	0	42.86	25.14	3.49	65.35	33.71	11.71	74.57	35	4.41	83	6900
6	V. H.	53	3. 5.	++	2.36	2.43	2.24	2.78	0.07	7.41	4.71	0	12.73	12.72	0	34.38	22.57	2.50	56.74	34.86	12.00	73.78	37	4.69	85	7600
7	L. A.	46	7. 6.	++	2.57	2.64	2.45	2.71	0.16	6.87	4.00	0	10.53	14.40	0	37.89	23.68	2.80	57.75	35.43	11.43	74·59	38	4.71	88	6900
8	T. E.	47	2. 3.	++	2.71	2.71	2.71	3.29	0.29	7.80	4.14	0.07	10.25	15.86	0	39.65	26.00	3.07	60.40	34.29	11.43	68.58	40	4.80	88	6600
9	H. U.	41	8. 5.	+	3.14	3.50	2.53	3.71	0.21	9.68	5.15	0	13.92	15.71	0	42.46	27.71	3.71	68.58	37.71	10.57	83.92	37	4.87	91	6400
10	T. Y.	39	24. 5.	+	3.00	3.35	2.45	5.00	0.29	12.36	4.57	0	11.72	17.72	0	45.44	30.29	3.64	71.97	40.57	11.43	86.15	39	5.00	90	8300
11	S. J.	43	4. 5.	+	2.86	3.35	1.99	3.14	0.14	8.47	4.70	0	13.06	12.98	0	36.06	23.68	3.49	59.57	35.43	9.72	81.14	36	4.37	84	5200
12	N. K.	52	18. 4.	+	2.93	3.07	2.74	3.07	0.28	6.92	6.86	0	16.33	17.43	0	41.50	30.29	3.35	67.49	42.29	11.71	84.52	42	5.12	95	8500
13	V. V.	48	2. 5.	+	2.21	2.35	2.04	3.49	0.28	7.42	5.16	0	11.47	16.56	0	36.80	27.42	2.63	57.72	34.29	9.14	65.03	45	5.47	91	8700
14	N. J.	28	13. 3.	+	2.79	3.07	2.42	3.63	0.35	7.98	4.72	0	10.98	16.00	0	37.21	27.14	3.42	58.58	33.71	10.00	65.14	43	5.08	90	8100
15	R. A.	29	9. 5.	+	2.44	2.86	1.91	4.12	0.14	9.18	3.58	0	8.14	13.86	0	31.50	24.00	3.00	50.73	34.29	10.00	65.20	44	5.12	95	5100
16	R. K.	29	29. 5.	+	2.93	3.07	2.73	2.77	0.21	6.45	4.30	0.07	10.39	17.71	0	43.20	27.71	3.35	62.76	33.14	11.14	64.80	41	5.00	90	5200
17	K. E.	48	12. 6.	+	2.57	2.79	2.30	3.13	0.07	6.89	3.16	0	7.02	15.71	0	34.91	24.57	2.86	51.11	34.00	10.86	62.28	45	5.60	99	7900
Mean (17)					2.74	2.96	2.39	3.31	0.20	8.04	4.48	0.03	11.28	15.79	0	39.75	26.31	3.19	61.16	35.61	11.01	73.11	40	4.92	90	7100
Healthy control persons (HELVE, 1946): Mean (42)					2.86	3.19	2.41	4.61	0.16	10.63	3.19	0.04	7.45	12.43	0.03	29.25	23.09	3.42	49.70	36.98	11.17	71.90	42.5	4.40	76	5730

RESULTS

Table I shows the results for the *blood phosphorus* fractions studied.

The content of *inorganic phosphorus* did not differ from those obtained for healthy individuals.

No abnormality was noted in the *pyro-* and *hexosephosphate-P* of the blood.

The amount of *diphosphoglyceric-acid-P* was in most cases slightly higher than the average in the control series, but the

TABLE II

PLASMA CHOLESTEROL AND PHOSPHOLIPIDS

(C = TOTAL CHOLESTEROL; CE = CHOLESTEROL ESTERS; TLP = TOTAL LIPIN PHOSPHORUS; ELP = ETHER-SOLUBLE LIPIN PHOSPHORUS; P = TOTAL PHOSPHATIDES [25 × TLP VALUE]; CE/C = RELATIVE PROPORTION OF ESTER CHOLESTEROL TO TOTAL CHOLESTEROL; P/C = RELATIVE PROPORTION OF PHOSPHATIDES TO TOTAL CHOLESTEROL.)

No.	C mg%	Ce mg%	Tlp mg%	Elp mg%	P mg%	Ce/C %	P/C %
1	146	92	8.56	8.42	214	63	147
2	154	105	8.00	7.42	200	68	130
3	194	—	8.00	7.86	200	—	103
5	219	127	11.14	10.82	279	58	127
6	199	109	10.00	9.50	250	55	126
7	141	94	9.85	9.50	246	67	174
8	—	—	9.42	8.56	236	—	—
9	178	100	10.28	9.88	257	56	144
10	131	90	9.14	8.85	229	69	175
11	148	92	9.00	8.40	225	62	152
12	154	100	7.72	7.58	193	65	125
13	141	99	9.14	8.84	229	70	162
14	—	—	7.00	6.85	175	—	—
15	119	75	8.56	8.14	214	63	180
16	168	99	8.42	8.14	211	59	126
17	226	140	8.70	8.14	218	62	96
Mean (14)	166	102	8.93	8.57	223	61	135
Healthy control persons (HELVE, 1947):							
Mean (13)	177	105	9.26	8.33	233	59	131
Lowest	113	66	7.43	6.86	186	43	76
Highest	246	166	11.00	9.71	275	106	243

difference was not so distinct as to allow any definite conclusions, considering the relatively small number of cases.

As regards the *total acid-soluble phosphorus* and *total phosphorus* no difference was observed between the patients with carbon monoxide poisoning and the healthy control persons.

Table II shows the figures obtained for *plasma lipids* in the patients with carbon monoxide poisoning. The cholesterol and ester cholesterol, as well as the lipid phosphorus, seem to vary within normal limits. The mutual relations of these lipids also reveal no abnormalities.

SUMMARY

Chronic carbon monoxide poisoning has been studied as affecting the inorganic phosphorus, pyrophosphate-P, hexosephosphate-P, diphosphoglyceric-acid-P, total acid-soluble-P, and total phosphorus of whole blood, plasma and erythrocytes; the series consisted of 17 cases. Attention was also directed to the cholesterol, ester cholesterol, total lipid phosphorus, and ether-soluble lipid phosphorus of the plasma. Comparison of the results with normal figures showed no clear differences.

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ANTIBIOTICS IN LICHEN

I

By

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Botanists and chemists have always been particularly interested in lichen. SCHWENDENER's (14) symbiosis theory, contested in its time especially by ELFVING (4), is now generally considered conclusive (5, 11, 18). According to it, a fungous and an algal component can always be distinguished in the lichen. On the other hand, the chemistry of lichen, in particular that of so-called lichen acids, has been zealously studied. These, in general very insoluble and well-crystallising compounds have not been found anywhere else in nature. As early as in 1907 ZOPF (20) was able to isolate 149 lichen acids.

After the impetus provided by penicillin and since the development of antibiotics, lichen was also subjected to studies from this point of view. The Americans BURKHOLDER and EVANS (3) published in 1944—45 the results of their tests performed on about one hundred species of American lichen, 52 of which were found to possess an unmistakable growth-inhibiting effect on either *Staphylococcus aureus* or *B. subtilis*, or both. Of the Gram-negative bacteria, *Alcaligenes faecalis*, *Proteus vulgaris*, and *Escherichia coli* suffered slight inhibition under the influence of some species of lichen. In 1946 BARRY (2) found that *diploicin* ($C_{16}H_{11}O_5Cl_3$) previously isolated (12) from the *Buellia canescens* had an inhibiting effect on the growth of diphtheria and tubercle bacilli in a dilution of 1: 100,000. MARSHAK (9) isolated in 1947 from the *Ramalina reticulata*

growing on the Western coast of North-America a crystalline compound ($C_{16}H_{14}O_6$) which specifically inhibited the growth of different tuberculous strains at titres of 1: 20,000—1: 50,000, and he found that it delayed the progress of human tuberculosis in guinea-pigs. The Swiss STOLL, RENZ and BRACK (16) found in 1947 that *usnic acid* ($C_{18}H_{16}O_7$) is an active substance in the majority of lichens. The *usnic acid* isolated by them from *Cetraria islandica* was able to arrest the growth of bacteria at the following titres: some TB-strains (*hominis*, *bovis*, *avum*) 1: 320,000—1: 800,000, *Mycobact. phlei* 1: 125,000, *Streptoc. pyogenes* 1: 100,000, *Staphyloc. aureus* 1: 100,000. *Usnic acid* appeared not to have any effect on Gram-negative bacteria nor on yeast nor Penicillium. They also found a similar effect in *vulpic*, *d-protolichesteric*, *lichesteric*, *dihydro-lichesteric*, *physodic*, and *diffractic* acids.

It is interesting that the use of lichen, especially in popular medicine, has been general since ancient times. In the 17th century Iceland moss (*Cetraria islandica*) was used in Central Europe as laxative, and since the beginning of the 18th century as a cough medicine relieving irritation (6); also in chest maladies, catarrhal hemoptysis (7) and boiled in milk particularly in tuberculosis (10). Similarly, according to an old Finnish tradition, reindeer-moss (*Cladonia alpestris*, *rangiferina* or *silvatica*) boiled in water with the lid on and drunk hot is used as a remedy against tuberculosis. Lung moss (*Lobaria pulmonaria*), cladonia (*Cladonia*) and the beard moss species (*Usnea* and *Alectoria*) are most commonly employed as cough remedies, In addition, the latter qualities are extensively used in the treatment of wounds and some eruptions (e.g. for bathing chafed toes) as well as in dysentery. Beard mosses have been put into water to wash a sick child. According to the Norwegian tradition *Peltigera aphtosa* is also used for healing skin diseases (8). The use of lichen in popular medicine is interesting, since many among the above-mentioned species of lichen contain acids which were proved active (*Cetraria islandica usnic*, and *protolichesteric* acid, all *Usnea* species, as well as *Cladonia alpestris* and *silvatica usnic* acid) (20) and since lichen acids in general (*usnic* acid always!) are found on the surface of the plants (19) and are therefore able to exercise their influence even if they are difficult to dissolve. — *Lichen islandicus* used also to belong to the medical remedies of the Finnish pharmacopeia but was omitted in 1915 (13) — probably as »ineffective».

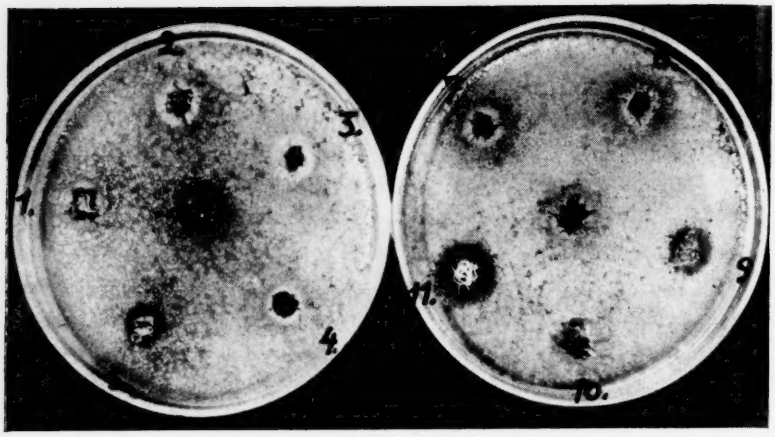

Fig. 1. — *B. subtilis*. Species of lichen: 1) *Parmelia physodes*, 2) *Parmelia sulcata*, 3) *Physcia stellaris*, 4) *Physcia pulverulenta*, 5) and 6) *Parmelia stenophylla*, 7) *Alectoria chalybeiformis*, 8) *Alectoria implexa*, 9) *Ramalina thrausta*, 10) *Usnea hirta*, 11) *Usnea sp.* 12) *Alectoria chalybeiformis*. Note strong growth around *Parmelia sulcata*, *Physcia*, and *Alectoria* species.

The Writer's Own Investigations. — The tests were begun in January, 1948, on the basis of the extensive popular use of lichen. The lichen species used for the tests were collected in January—October, 1948, in the vicinity of Helsinki, at Elimäki, Tyrväntö, and Laukaa. They were stored in the ordinary way, dried in paper bags. The tests were made with each lichen species[1] on ten bacteria: *Sarcina aurea*, *Staphyloc. aureus*, *Streptoc. β-hemolyticus*, *Escherichia coli*, *Proteus vulgaris*, *Hemophilus pertussis*, *Corynebact. diphteriae*, *B. subtilis*, *B. megatherium* and *Pseudomonas pyocyaneus*. From a broth culture after a growth of 24 hours a subculture was made on Petri's dishes. The surface of the latter having dried, lichen pieces with an average weight of 5 mg, pounded with a sterile puncher of 5 mm diameter, were placed on the dish. The reading of the results took place in 1 to 3 days, according to the rapidity of the growth of the bacteria. + = antibiotic area counted 1 to 7 mm from the margin of the lichen, ++ = antibiotic area surpassing 7 mm, (+) = bacterial growth weakened around the lichen, — = no response (Table 1).

[1] The species were determined by Professor E. HÄYRÉN, to whom I am also grateful for his excellent advice with regard to the collecting of lichen.

TABLE 1

EFFECT OF PIECES OF LICHEN ON VARIOUS BACTERIA. + = ANTIBIOTIC AREA 1—7 MM WIDE COUNTED FROM MARGIN OF LICHEN, ++ = OVER 7 MM, (+) = BACTERIAL GROWTH WEAKENED, NO CLEAR RING, — = NO EFFECT. U = LICHEN CONTAINS USNIC ACID, A = ATRANORIN, P = PHYSODIC OR PHYSODALIC ACID

		Sarcina aurea	*Staphyloc. aureus*	*Streptoc. β-hemol.*	*Escherichia coli*	*Proteus vulgaris*	*Corynebact. diphteriae*	*B. subtilis*	*B. megatherium*
Sphaerophorus fragilis ..		++	+	+	—	+	—	+	+
Peltigera aphtosa		—	—	—	+?	—	—	—	+?
» polydactyla ..		—	—	—	—	—	—	—	—
» canina		—	—	—	+?	—	—	—	+?
Nephroma parilis		—	—	—	—	—	—	—	—
» resupinatum ..		—	—	—	—	—	—	—	—
Cladonia alpestris	U	+	—	+	—	—	—	+	+
» silvatica	U	++	+	—	—	—	+	++	+
» carneola	U	++	+	+	—	—	+	++	+
» uncialis	U	++	+		—	—	—	+	++
» deformis	U	++	+	+	—	—	+	++	++
» coccifera	U	++	+	+	—	—	+	++	+
» amaurocrea	U	++	+	+	—	+	+	++	+
» rangiferina	A	—	—	—	—	—	—	—	—
» turgida		—	—	—	—	+?	—	—	—
» cariosa		—	—	—	—	—	—	—	—
» bacilliformis ..		—	—	—	—	—	+	++	++
» gracilis	A	—	—	—	—	—	—	—	+
» squamosa		—	—	—	—	—	—	—	+
Stereocaulon paschale ..	A	+	+	—	—	+	—	+	+
» sp.	(A)	—	—	—	—	+	—	+	+
Gyrophora polyphylla ..		—	—	—	+?	—	—	—	—
» hyperborea ..		—	—	—	—	-	—	—	—
» deusta		—	—	—	—	—	—	—	—
» polyrhiza		—	—	—	—	—	—	—	—
Pertusaria amara		+	+	+	—	—	—	+	+
» sp.		+	—	+	—	—	—	+	+
Haematomma ventosum	U	++	+	+	—	+	—	+	+
Lecanora varia	U	+	—	—	—	—	+	++	+
» subfusca (allophana)	A	—	—	—	—	—	—	—	—
» caenisea	A	+	—	—	—	-	—	—	+
» carpinea		—	—	+?	—	—	—	—	—
Ochrolechia tartarea		—	—	—	—	+	—	+	+
Cetraria islandica	(U)	+	+	+	+	+	—	—	+
» tenuifolia	A	—	—	—	+	+	—	—	—
» aculeata		+	(+)	+?	—	—	—	—	+
» chlorophylla ..	A	—	+	+	—	+	—	+	+
» pinastri		++	+	+	+?	+	+?	++	+
» glauca	A	—	—	—	+	+	-	—	—

TABLE 1 (Continued)

		Sarcina aurea	*Staphyloc. aureus*	*Streptoc. β-hemol.*	*Escherichia coli*	*Proteus vulgaris*	*Corynebact. diphteriae*	*B. subtilis*	*B. megatherium*
Parmeliopsis ambigua ..	U	++	+	+	+	+	—	+	+
Parmelia physodes	A,P	+	+	—	—	—	—	+	+
» tubulosa	A,P	+	+	—	—	+	+	—	+
» sulcata	A	+	—	—	—	—	—	—	—
» centrifuga		+	+	(+)	—	—	—	+	+
» saxatilis	A	—	—	—	—	—	—	—	—
» incurva	U	++	+	(+)	—	+	—	+	+
» conspersa	U	++	—	—	—	—	—	+	+
» stenophylla	A	+	—	+	+?	+?	+	+	+
» olivacea		—	—	—	—	—	—	—	+
» stygia		—	—	—	—	—	—	—	—
» *fuliginosa*		—	—	—	—	—	—	—	—
» exasperatula ..		—	—	—	—	—	—	—	—
» *sorediata*		+	+	—	+	+	+?	+	—
Evernia furfuracea	A,P	+	+	+	—	—	—	+	+
» prunastri	A,U	++	+	+	—	—	—	++	+
Usnea hirta	U	++	+	+	—	—	+	++	++
» comosa	U	++	+	+	—	—	—	+	+
» *dasypoga*	U	++	+	+	—	—	+	++	+
» glabrescens	U	++	+	+	—	—	—	++	+
» sp.		++	+	+	—	—	—	++	+
Alectoria implexa	A	—	—	—	—	+	—	—	—
» chalybeiformis..		—	—	—	—	—	—	—	—
Ramalina farinacea	U	++	+	—	—	—	+	++	+
» fraxinea	U	++	—	—	—	—	—	++	+
» populina	U	+	+		—			+	
» obtusata	U	++	+		—	+	+?	++	+
» thrausta	U	++	+	+	—	—	—	++	+
Xanthoria parietina		—	—	—	—	—	—	—	—
Physcia stellaris	A	—	—	—	—	+	—	—	—
» aipolia	A	—	—	—	—	—	—	—	—
» tribacea	A	—	—	—	—	—	—	—	—
» obscura (ciliata)	A	—	—	—	—	—	+	—	—
» pulverulenta	A	—	—	—	—	—	—	—	—
» ascendens	A	—	—	—	—	—	—	—	—
» tenella		+?	—	—	—	—	—	—	—
» caesia	A	+	+	(+)	—	++	—	+	+
Anaptychia ciliaris	A	—	—	—	—	—	—	—	—
Crogynia membranacea ..		+	—	+	—	—	—	—	—
Pannaria brunnea		—	—	—	—	—	—	—	—
Caloplaca aurantiaca		—	—	—	—	—	—	—	—
Phlyctis agaelea		—	—	—	—	—	—	—	—
82. Lepraria flava		+	—	+	+	+	—	+	—

TABLE 2

DISH TESTS WITH TWO ISOLATED LICHEN ACIDS. THE FIGURES ILLUSTRATE WIDTH OF ANTIBIOTIC AREA COUNTED FROM EDGE OF FILTER-PAPER DISC IN CM. (+) = GROWTH WEAKENED

	Sarcina aurea	Staphyloc. aureus	Streptoc. β-hemol.	E. coli	Proteus vulgaris	C. diphteriae	B. subtilis	B. megatherium
Usnic acid sodium chloride, 1% glycol solution	0.9	0.2	(+)	(+)	(+)	0.3	0.8	0.7
Crystalline derivate of atranorin, $NaHCO_3$-solution	0.3	0.2	(+)	0.1	0.5	?	(+)	(+)

The first observation made in the tests was that in the majority of the lichen species taken direct from nature, no micro-organisms grow even under the most favourable conditions, in other words they are practically sterile.

Of the 82 tested lichen species, 22 contain usnic acid (20). These species designated by the letter U had a clearly inhibiting influence on *Sarcina*, *Staphylococcus*, *Streptococcus*, *B. subtilis* and *B. megatherium* and in comparatively numerous cases on *Diphtheria* bacilli, but they did not affect the Gram-negative rods. A similar result was obtained with the *crystalline l-usnic acid* isolated from *Cladonia alpestris* (15) (Table 2). The tests were made to correspond to the lichen tests by saturating a filter-paper disk with a diameter of 11 mm with usnic acid sodium chloride 1 per cent glycol solution. The usnic acid content in lichen being on an average about 1 per cent, the tables are comparable to each other. The isolated acid also had slight inhibiting influence on growth of Gram-negative rods. An effect similar to that of usnic acid could also be brought about with *Parmelia physodes*, *Parmelia tubulosa* and *Evernia furfuracea*, whose active substance are evidently *physodic* and *physodalic* acids (marked on the table with P).

Of the 20 lichen species inhibiting the growth of *Proteus vulgaris*, 9 contain *atranorin* ($C_{19}H_{18}O_8$) (A on the table), *ramalina obtusata obtusatic* acid ($C_{18}H_{18}O_7$), *cladonia amaurocrea coccell* acid ($C_{20}H_{22}O_7$), *Haematomma ventosum divaricatic* acid and *Sphaerophorus fragilis sphaerophorin*. All these acids are 1—3-dioxydepsides and closely

TABLE 3

L-USNIC ACID SODIUM CHLORIDE SOLUTION SERIES IN KIRCHNER'S NUTRIENT MEDIUM. CULTURES WITH TWO TB-STRAINS

	1: 10,000	1: 15,000	1: 20,000	1: 30,000	1: 40,000	1: 60,000	1: 80,000	1: 120,000	1: 160,000	1: 240,000	1: 320,000	Control	Control
TB-strain 2132	—	—	—	—	—	—	±	+	+	+	+	+	+
							+	+	+	+	+	+	+
							+	+	±	+	±	+	+
										+	+	+	+
TB-strain 2247	—	—	—	—	—	—	+	±	+	+	+	+	+
							+	+	+	+	+	+	+
										+	+	+	±

related as to their structure (1). In *Lepraria flava* and *Cetraria pinastri* the active substance is probably *vulpic* acid with its derivates. No fully evident action on any of the bacteria used in the tests was found with *atranorin* extracted from *Stereocaulon paschale*, which may be explained by the circumstance that it could not be made dissolvent in a satisfactory way. On the other hand, a crystalline derivate of *atranorin* obtained by distillation of chloroform-alcohol solution produced an unmistakably inhibiting effect on *Proteus*, a somewhat weaker one on *E. coli*, *Sarcina*, *Staphylococcus* and *B. subtilis* (Table 2). The substance prevented the growth of *Proteus* in a solution of 1: 10,000.

Escherichia coli seems in general to be inhibited by the same species of lichen as *Proteus vulgaris*, but the effect is weaker. The lichen under examination were not found to possess any activity with regard to *Hemophilus pertussis* and *Pseudomonas pyocyaneus*.

When experimenting with the l-usnic acid sodium chloride solution on two TB-strains, an inhibitive effect was obtained at the titres 1: 60,000, and a weakening of the growth at titres of 1: 160,000 (Table 3).

In preliminary tests with pathogenic and other fungi the species *Cetraria glauca*, *Parmelia stenophylla*, *Evernia prunastri*, *Usnea dasypoga* and *Alectoria sarmentosa* were found to act on *Actinomyces*

sulfuroides, *Trichophyton farineculatum*, *Trichophyton interdigitalis* and *Epidermophyton inguinale*.

In the *B. subtilis* dishes there was, in addition to an inhibitive effect of several lichen species, also a clear influence promoting the bacterial growth. (Fig. 1). This satellite phenomenon has not been more closely investigated so far.

SUMMARY

Of 82 lichen species 52 were found to affect some bacteria under examination. There is a certain rough parallelism between the action of the lichen and its content of different lichen acids. The growth-inhibiting effect of usnic acid on TB established by STOLL *et al.* is confirmed.

A crystalline derivate of atranorin appeared to have a specific effect on *Proteus vulgaris*, preventing its growth at 1: 10,000. Some lichen species were found to possess activity with regard to pathogenic fungi. A satellite phenomenon is ascertained between *B. subtilis* and several lichen species.

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THE VALUE OF THE SALT DISPERSIBILITY TECHNIQUE OF KAHN IN THE EVALUATION OF SERO-DIAGNOSTIC TESTS FOR SYPHILIS

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The need for good tests in evaluating positive serologic findings in persons without any clinical signs or history indicating syphilitic infection is ever increasing. The most promising test hitherto seems to be the Euglobulin-inhibition test introduced by NEURATH *et al.* 1947 (3). In 1948 NEURATH (2), however, states that the serologic analysis has not yet reached the stage where it will take the place of clinical examination and probably never will. So all known tests seem to have their limitations and further study of the known tests is indicated.

Of the different »verification test» techniques published by KAHN the differential temperature technique seems to be the only one which has been sufficiently studied. According to REIN and ELSBERG (5) the correlation of the syphilitic type of »verification reaction» to syphilitic infection is not apparent and also non-syphilitic individuals occasionally gave the syphilitic type of the »verification reaction». The present paper reports the results obtained with KAHN's salt dispersibility technique. This test seems to be technically the simplest to carry out in the laboratory routine so that it would be of great importance if its value could be established.

Technique. — According to KAHN's in 1943 published paper (1) we have used a 30 per cent solution of sodium chloride. The KAHN test having been performed and the results read in the usual way (4), 0.1 and 0.15 ml of the 30 per cent salt solution were added, respectively, to tubes with antigen amounts of 0.0125 and 0.025 ml; the rack was shaken by hand for ten seconds, placed in the 37° C. water bath for five minutes, again shaken for ten seconds, and the tubes examined to find if the precipitates have been totally or partially dispersed or increased. According to KAHN, the precipitates usually are dispersible in non-syphilitic sera and not dispersible or even more distinct in syphilitic sera. We used two-tube tests and readings +++, ++, +, ± and —. Reading +?, sometimes used in this laboratory, has been considered equal to + and reading —? to —.

Materials. — The salt dispersibility technique of KAHN has been applied without any selection to 3,424 sera which were more or less positive in KAHN test. These were the positive sera of 48,566 specimens which were tested in our laboratory during the ten months of our work. The cases with reactions reported as —? are included in these Kahn-positive cases.

Results. — The adding of 30 per cent salt solution caused a change in the result of the test in 368 cases (10.7 per cent). The precipitates were increased in 164 cases (45 per cent) and decreased in 204 cases (55 per cent). Of the above mentioned 368 cases we collected the available data, concerning the possible syphilitic infection, of 230 cases chosen at random. Of these 230 cases 114 showed an increase of the precipitates in the test and 116 a decrease. In Tables 1 and 2 the diagnoses of the above mentioned 230 cases are presented.

Repeated Tests from the Same Persons. — In comparing the results of the salt dispersibility technique using successive serum specimens from 40 persons whose first serum had shown a change we observed the following: a) A change in the same direction with successive sera collected from 20 patients. b) A change was no longer observed with sera from 12 cases. c) The change was in the opposite direction with the sera from 8 cases.

Discussion. — In comparing Tables 1 and 2 the conclusion is not easy to escape that different types of syphilis as well as the probably non-syphilitic cases, are equally represented in two groups

TABLE 1

THE DIAGNOSES OF 114 CASES IN WHICH THE SALT DISPERSIBILITY TECHNIQUE SHOWED A RESULT OF THE »SYPHILITIC TYPE»

TABLE 1

THE DIAGNOSES OF 114 CASES IN WHICH THE SALT DISPERSIBILITY TECHNIQUE SHOWED A RESULT OF THE »SYPHILITIC TYPE»

Group [1]	Syphilis								Insuffic. studied cases	Probably [2] non-syphil. cases	Total number of cases
	Primary	Secondary	Early latent	Cardiovasc.	Neurologic	Congenital	Not defined	Total			
1	1	15	21	4	10	1	6	58	13	5	76
2	1	3	5	1	5	4	3	22	11	2	35
3	—	1	1	—	1	—	—	3	—	—	3
	2	19	27	5	16	5	9	83	24	7	114

TABLE 2

THE DIAGNOSES OF THE 116 CASES IN WHICH THE SALT DISPERSIBILITY TECHNIQUE SHOWED A RESULT OF THE »NON-SYPHILITIC TYPE»

Group [1]	Syphilis								Insuffic. studied cases	Probably [3] non-syphil. cases	Total number of cases
	Primary	Secondary	Early latent	Cardiovasc.	Neurologic	Congenital	Not defined	Total			
1	3	5	13	2	15	3	5	46	20	7	73
2	2	3	7	2	5	—	—	19	9	2	30
3	1	2	2	—	2	1	2	10	1	2	13
	6	10	22	4	22	4	7	75	30	11	116

[1] Because the estimation of precipitates is subject to variations especially when changes are minutes, we have presented our results in three groups according to more or less marked change. So group one represents those cases in which the adding of 30 per cent salt solution has changed the report of the summarised result of the two-tube test only slightly *e.g.*, from + to ++ or conversly. In group two are presented the cases in which the change has been marked *e.g.*, from + to +++ or conversly and in group three those in which the change has been very marked, *e.g.*, from ± to +++ or conversly.

[2] The diagnoses of the »probably non-syphilitic» cases in Table 1 were *Pleuritis exsudativa*, *Bronchitis acuta*, *Spina bifida occulta*, *Endocarditis lenta* in two cases and *Commotio cerebri* in two cases.

[3] The diagnoses of the »probably non-syphilitic» cases in Table 2 were *Pneumonia interstitialis*, *Bronchitis acuta*, *Febris e causa ignota* (*Malaria?*), *Malaria* in five cases and *Bronchopneumonia* in three cases.

of cases where the salt dispersibility technique has given a result of »syphilitic» or »non-syphilitic» type. It is to be mentioned, however, that the non-syphilitic nature of the cases in the group of dispersible precipitates seems to be more definite. The results with successive sera from the same persons confirm the idea that results of the salt dispersibility technique are not well correlated with the syphilitic or non-syphilitic nature of the cases. According to the results presented in Table 2 even among those sera where the precipitates were most completely dispersed (group 3) the majority were definitely of syphilitic origin. From the results described it can be concluded that the salt dispersibility technique of KAHN is not of great value in detecting misleading positive results of sero-diagnostic tests, not even when applied to typing of sero-positive cases that are free from clinical indications of syphilis.

The phenomenon of the dispersibility of the precipitates after increasing the ionic strength of the solution is, however, in some cases very marked and easy to repeat. It may suggest that the precipitates are produced in different sera by different types of serum proteins or that the »syphilitic antibody» is not always in the same protein fraction of the serum. The phenomenon requires further study.

Summary. — The salt dispersibility technique of KAHN in detecting misleading positive results of sero-diagnostic tests seems not to be of great value according to the investigation presented in this paper.

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ON THE ANOXIC SUPPRESSION OF THE GASTRIC SECRETORY FUNCTIONS

By

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It has been known since BAYEUX (2) that both the volume and the acidity of gastric secretion, as induced with a standard stimulus, is decreased at high altitudes. This suppression is due to anoxia, and not essentially to a concomitant alkalosis (8). In previous investigations attention has been paid primarily to the volume and acidity of the secrete (2, 3, 10, 20, 15, 9, 17, 8). The gastric juice, however, has also several other components. SLEETH & VAN LIERE (16) and PICKETT & VAN LIERE (14) included chloride determinations in their experiments. On the behaviour of pepsin — and of the inorganic components of gastric juice other than the chlorides — in anoxia hardly any information could be found. GIANOTTI & GOLDBERGER (6), in the course of a mountaineering excursion, observed that the secretion of pepsin also had been suppressed, but they attributed this effect to the heavy exercise performed.

It was thought desirable to study whether in anoxia the secretion of HCl is selectively suppressed, or whether there occurs a parallel decrease in the secretion of some of the other components, too. For this purpose — in a series of experiments in which human subjects were exposed to a low atmospheric pressure — the gastric concentrations of pepsin, of total chlorides, and of calcium were determined, in addition to the titratable acidity, pH, and the volume of the samples. The determination of calcium was included

in the programme because it has been supposed to give a good quantitative indication of the proportion of the non-HCl components of the juice (7).

In the earlier investigations, various stimuli were used. BAYEUX and HELLEBRANDT *et al.* administered a test meal, SLEETH & VAN LIERE water, and alcohol per os was used by HELLEBRANDT *et al.*, by WARREN, by HARTMANN *et al.*, by STÄMPFLI & ENDTNER, and by HARTIALA & KARVONEN. GIANOTTI & GOLDBERGER, DELRUE, and HELLEBRANDT *et al.* used histamine, and SELMANOVA a caffeine test meal.

The injection of vagomimetic drugs is known to produce, through a peripheral action, a secretory response very similar to that obtained by sham feeding (18, 12). Stimulation with a vagomimetic drug has not been so far tried under anoxic conditions. In the present work, a slowly acting vagomimetic drug, carbaminoylcholine chloride, was chosen as one of the two stimuli used. It has been previously shown to stimulate the gastric secretion in dog and man (13, 4). As administered as an injection it also shares with histamine the advantage of obviating the addition of any extraneous fluids as stimuli into the stomach; thus it enables one to examine the pure gastric secrete. In addition, a parallel series of tests using alcohol as a stimulus was also carried out on the same subjects.

METHODS

The series was performed on three healthy medical students, A, E, and O. A few additional experiments were made on an achlorhydric but otherwise healthy student, Y. The tests were made in the morning, about 1 to 2 hours after awakening, on an empty stomach.

After swallowing the stomach tube the gastric contents were emptied four times, at the intervals of 10 minutes. After this, the stimulus was applied. Two stimuli were used, either the administration of 300 ml of 5 per cent alcohol, dyed with methylene blue, or the subcutaneous injection of 0.25 mg of carbaminoylcholine chloride (»Carbachol», May & Baker). In the alcohol experiments, 10 ml samples were drawn at 10 min. intervals until 90 min. had elapsed after the administration of the stimulus; then all the available fluid was drawn and measured. In the other series, all the gastric contents were collected at 10 min. intervals. The volume of the samples was measured.

The pH of the samples was measured with a glass electrode and a valve potentiometer ('Radiometer').

The titratable acidity was determined in the usual way, using phenolphtalein as an indicator; the results are expressed as ml 0.1 N alkali used *pro* 100 ml of gastric contents.

The dilution of methylene blue in the alcohol experiments was determined with the aid of a Havemann type photoelectric colorimeter; the samples were cleared with centrifuge, and an orange filter was used for the determinations.

The calcium determinations were carried out according to the titrimetric method of KRAMER & TISDALL (11).

The total chloride concentration was estimated after a nitric acid digestion, using the modification of WILSON & BALL (21) of VAN SLYKE's method (19).

For the pepsin determinations a slightly modified ANSON & MIRSKY (1) technique was adopted. 2.5 ml of a standard ox haemoglobin substrate was digested at 35.5° C for 5 minutes with 0.5 ml of a 1: 2 dilution of the original sample. The amount of protein hydrolysis products was measured colorimetrically, after adding FOLIN & CIACOLTEAU's (5) reagent. As a standard of the colorimeter readings, an extinction curve for tyrosine solutions of known strengths was made. The unit of pepsin was defined strictly in accordance with ANSON & MIRSKY. A dilution series was made of a fairly concentrated sample of pepsin, and the relation of the strength of the pepsin solution to the concentration of the resulting digestion products was assessed. With the fairly high pepsin concentrations used this relation proved not to be linear. An empirical dilution graph was thus accepted as the basis of the estimation of the pepsin concentration. In control experiments it was ascertained that the colour reaction given by the gastric contents and the reagents without digestion was insignificant, of the order of 0.1 pepsin units per ml of gastric contents. Therefore, no attempt was made to exclude this small source of error. In duplicate determinations (5 duplicate digestions) the agreement was very good; the maximum difference was 3.0 per cent and the average difference was 2.0 per cent.

The anoxia experiments were performed in a low pressure chamber. Each subject had to perform the following experiments:

(1) a 'training' test at sea level — the results of which were neglected — with either alcohol or carbaminoylcholine as a stimulus;
(2) and (3) alcohol tests at sea level;
(4) and (5) alcohol tests at the simulated altitude of 5000 m;
(6) and (7) carbaminoylcholine tests at sea level;
(8) and (9) carbaminoylcholine tests at 5000 m.

The altitude of 5000 m. was chosen, as this height was for one of the subjects (E) the maximum at which he remained conscious. The experiments were performed in varying order, in the course of four months.

RESULTS

A. CARBAMINOYLCHOLINE EXPERIMENTS

Amount. — At sea level, all the three normal subjects responded to the injection of carbaminoylcholine with a gastric secretion. The secretory response showed great variations between each experiment. The volume of the secrete collected during 90 minutes after the administration of the drug was on the average 104 ml. The secretory response at the simulated altitude of 5000 m was much less — if anything at all. The average volume was correspondingly only 19 ml. The amounts collected in each experiment are shown in Table I. An average has also been calculated for the volume of the secrete collected at each 10 min. interval; the results are expressed in Fig. 1. However, the scatter of the individual values was great.

pH. — At sea level, the secrete was in all experiments fairly acid. In Fig. 2, the average pH of the samples has been plotted against

TABLE I

TOTAL AMOUNTS OF GASTRIC JUICE, 0.1 NORMAL ACID AND PEPSIN COLLECTED WITHIN 90 MINUTES AFTER ADMINISTERING 0.25 MG OF CARBAMINOYLCHOLINE CHLORIDE

Subject	Volume ml.	0.1 N Acid ml.	Peusin Units
Sea level:			
A	134	32	381
	79	20	252
E	157	106	925
	43	14	87
O	125	50	433
	83	40	465
Average	*104*	*42*	*424*
5000 m:			
A	12	3.0	32
	35	12.2	68
E	2.0	<1.0	7.7
	21	4.5	7.9
O	38	7.2	78
	8.5	2.7	19
Average	*19.4*	*4.9*	*35.4*

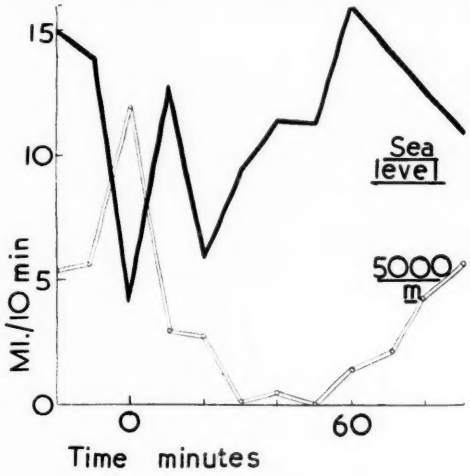

Fig. 1. — Carbaminoylcholine tests: the average volume of the secrete collected at each 10 min. interval.

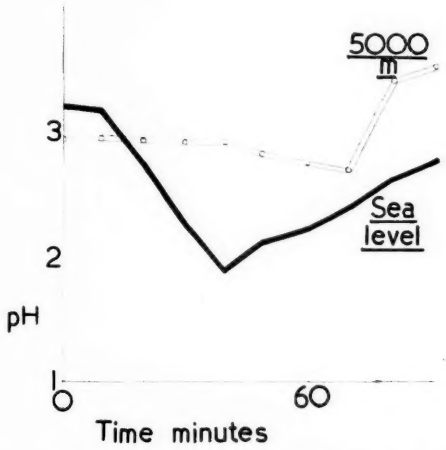

Fig. 2. — Carbaminoylcholine tests: the average pH of the samples as plotted against the time.

the time. The secretory response was first manifest in the samples taken 20 min. after the injection. At 5000 m, no clear tendency to any change was seen in the pH. The averages and the standard deviations were calculated for the pH of every sample taken from the 20th until the 90th minute of the test. The figures are given in Table II, together with the range of the variation. The difference

TABLE II

THE AVERAGE pH, AND THE AVERAGE CONCENTRATIONS OF ACID, OF PEPSIN, AND OF Ca AFTER STIMULATION WITH CARBAMINOYLCHOLINE

	pH 10—90 min.	Titratable Acidity ml of 0.1 N Acid per 100 ml 10—90 min.	Ca mg.% 0—90 min.	Pepsin Units per ml 0—90 min.
Sea level:				
Average	2.30	39	6.0	4.08
Standard deviation	±0.76	±19	±4.0	±1.50
Extreme values	5.30—1.60	8—80	1.3—18.8	0.32—6.04
5000 m:				
Average	3.13	28	6.6	2.04
Standard deviation	±1.82	±11	—	±0.94
Extreme values	7.10—1.60	12—50	1.0—44.4	0.36—4.35

between these averages (one pH unit) is, however, not statistically significant, thus indicating the great scatter of the individual experiments.

Titratable Acidity. — For the titratable acidity the corresponding figures are also given in Table II. Again, the difference between the means (11 ml. 0.1 N acid per 100 ml.) is not statistically significant. The average course of the concentration of acid after the stimulation mirrors that of the pH, both at sea level and at 5000 m. When the total amounts of acid, collected within 90 min. after the stimulation, are expressed as ml 0.1 N acid, a great difference in the acid secretion becomes manifest (Table I). At sea level, the secreted amounts of acid are on an average nine times more than at 5000 m.

Chloride. — The results of the chloride determinations show, that one of the subjects, A, never had a Cl concentration higher than 100 milliequivalents per litre, whereas it in subjects E and O often exceeded this limit. For A the average Cl concentration at sea level was 66 ± 11 millieq., and the range of variation was from 50 to 87 millieq. per litre (15 determinations). At 5000 m the corresponding figures were: average 70 ± 12 millieq., range from 56 to 90 (10 determinations). For the subjects E and O at sea level the average was 129 ± 9 millieq., and the total range of

variation was from 106 to 141 millieq. per litre (36 determinations). At 5000 m the corresponding average was 105 ± 11 millieq., and the range was from 89 to 119 millieq. per litre (11 determinations). The concentration of chloride was not affected significantly by anoxia in subject A, whereas in subjects E and O, who had a positive gastric juice — plasma gradient (more Cl in gastric juice than in plasma), the chloride concentration was distinctly lowered at 5000 m. The question remains whether the drop in the chloride concentration was parallel to a decreased HCl-secretion or whether

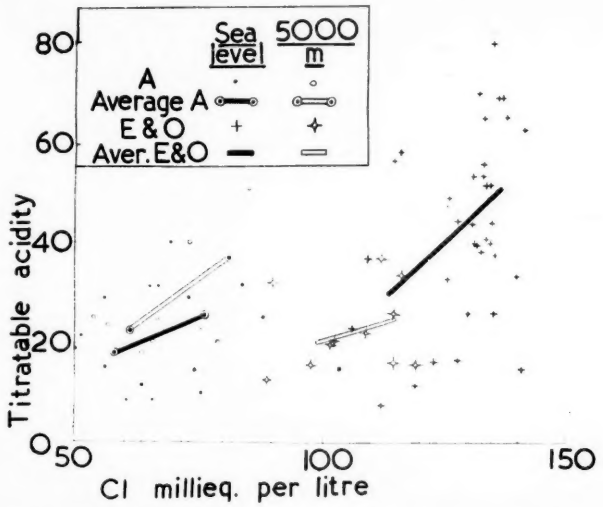

Fig. 3. — CarLaminoylcholine tests: the relation of the titratable acidity to the chloride concentration. The values of subject A and of subjects E & O are treated separately.

it occurred independently of it. In subject A, there existed a positive correlation of + 0.43 ± 0.16 between the titratable acidity and the chloride values. In subjects E and O, the corresponding correlation was + 0.55 ± 0.10. Evidently, the chloride values varied to a great extent parallelly with the acidity of the juice. More light to the relation of the titratable acidity and the chloride concentration is gained graphically in Fig. 3. Two pairs of lines have been drawn to indicate the average relation between the two sets of values, one of the pairs for A and the other for E and O. The lines are located according to the average distribution of the values. The graphs for sea level and 5000 m are in both cases so

TABLE

THE AVERAGE AND THE RANGE OF THE RESULTS OF THE ALCOHOL

	Level	Pre-experim.	Minutes after Admin		
			10	20	30
Intensity of colour per cent of that of the alcohol stimulus	Sea	—	63 —91—105	75— 90—106	42— 77—9
	5000	—	42— 71—96	94—107—130	38— 88—1
pH	Sea	6.9— 3.1—1.5	6.7—3.4—2.4	4.9— 2.9—2.3	4.2—2.5—1
	5000	7.2— 4.2—2.1	6.3—3.8—2.2	3.8— 3.0—2.6	3.9—3.0—2
Titratable acidity	Sea	7— 37—80	5— 11—18	5— 11—18	8— 20—4
	5000	1— 18—36	3— 5—7	6— 10—16	6—13—19
Cl millieq. per litre	Sea	65—106—132	1— 20—31	16— 28—41	19— 39—7
	5000	55—100—139	0— 17—27	8— 20—26	15— 25—3
Ca mg%	Sea	1.2— 6.4—18.2	0.1—1.6—3.0	0.1— 1.1—3.4	0.2—1.9—3
	5000	1.2— 9.2—41.5	0.2—1.1—1.8	0.1— 1.2—2.1	0.1—1.4—3
Pepsin units per ml	Sea	0.0— 3.2—6.0	0.1—0.9—1.5	0.3— 1.2—2.2	0.4—1.9—4
	5000	0.1— 2.5—4.7	0.1—0.4—0.8	0.5— 1.0—1.9	0.6—1.2—1

close to each other and they have so nearly the same slope that a parallel effect of the anoxia on both the acidity and the chloride concentration of the gastric juice is made most probable. This conclusion is borne out by the statistical treatment of the values. The regression coefficients for sea level and 5000 m do not differ significantly.

Calcium. — At sea level, the calcium values showed a considerable range of variation (Table II). There was a significant negative correlation (-0.44 ± 0.12) between the titratable acidity and the Ca-values; that is, the calcium concentration was the higher the more the non-HCl components contributed to the total secretion. At 5000 m, however, the variability of the calcium values was very great indeed, and there was no sense in calculating the standard deviation for this set of values, as their distribution was highly asymmetrical. There was also no correlation between the titratable acidity and the calcium values. It suggests that the secretion of all the non-HCl components was not suppressed proportionally.

Pepsin. — The mean pepsin concentration of the samples is shown in Fig. 4. The concentration at 5000 m was on an average half of that at sea level. No clear-cut tendencies were observed

III

TESTS; THE OBSERVED CONCENTRATIONS IN THE GASTRIC CONTENTS

ering the Alcohol						
40	50	60	70	80	90	
—70—88	24— 53—78	17— 43—66	10— 36— 61	9— 35—50	7— 32—48	
—78—99	18— 69—96	9— 62—91	9— 54—75	8— 49—73	3— 41—65	
—2.2—1.7	2.4—2.1—1.7	2.6—2.0—1.7	2.8—2.0—1.3	2.4—1.9—1.7	2.6— 1.9—1.7	
—2.6—2.2	3.6—2.6—2.1	3.7—2.5—2.0	4.0—2.4—1.9	4.2—2.3—1.6	4.6— 2.4—1.6	
— 23 —48	20— 33—50	16— 33—50	10— 30—55	28— 43—55	21— 41—58	
— 15 —25	15— 19—25	13— 20—29	19— 27—38	18— 30—42	10— 29—46	
—55—102	50— 69—105	55— 78—101	59— 86—111	63— 96—117	66—102—122	
— 27—38	34— 40—48	37— 49—57	49— 60—78	41— 65—90	61— 76—94	
—2.8—6.2	0.7—3.2—7.1	0.2—3.4—8.0	0.2—3.7—6.8	0.3—3.1—5.7	0.3— 3.8—6.0	
—1.5—3.4	0.2—1.6—3.4	0.7—2.0—3.5	0.2—2.1—3.6	0.2—2.2—4.2	0.2— 2.4—6.4	
—2.2—5.5	1.2—2.7—5.7	1.8—3.2—5.9	1.1—3.3—6.2	1.6—3.3—4.2	1.8— 3.7—5.1	
—1.4—2.1	1.0—1.5—2.0	0.8—1.6—2.0	1.2—1.9—2.3	2.0—2.3—2.9	2.1— 2.5—3.6	

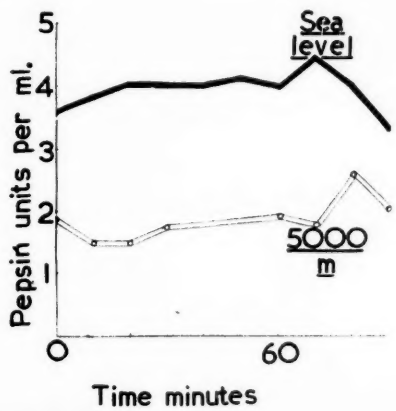

Fig. 4. — Carbaminoylcholine tests: the average pepsin concentration of the samples as plotted against the time.

during the experiments. The averages were calculated for all the pepsin concentrations in the samples taken within 90 minutes after the adiministration of the stimulus; they are shown — with the range of the variation — in Table II. The total output of pepsin is expressed in Table I. As for the secretion of pepsin, the difference between sea level and 5000 m seems to be more marked than for the inorganic components of the juice.

B. ALCOHOL EXPERIMENTS

The use of an indicator of the dilution — like methylene blue — has two aims: first, to indicate by the dilution the amount of the secrete; second, to make it possible to calculate the concentration of the different components in the pure secrete. The first aim can be invalidated already by variations in the rate of emptying of the stomach; the second purpose can be achieved only if the dilution indicator is not adsorbed by the mucous membranes. Methylene blue evidently does not fulfil this requirement. This was especially marked in the experiments at 5000 m. Quite regularly the concentration of the dye was higher in the samples taken 20 minutes than in those taken 10 minutes after the administration of the stimulus (Table III). The concentration of the dye often exceeded that in the administered fluid. These findings can only be explained by assuming that at the almost neutral pH immediately following the administration of the fluid a considerable portion of the dye was adsorbed by the gastric mucosa, whereas later on, at a lower pH, some of the adsorbed dye was slowly brought again into the solution, which now had a smaller volume. This irregularity was not so marked at sea level, which probably is connected with the more profuse secretory activity at normal barometric pressure.

Because of the inadequacy of the dilution indicator method there is no reason for giving a detailed mathematical treatment to the experimental results thus obtained. Still, there is some interest in the general tendencies observed (Table III). The rate of dilution was slower at 5000 m than at sea level. On an average, the 50 per cent dilution was passed at sea level between 50 and 60 minutes, whereas at 5000 m almost 80 minutes were required for this. A similar difference was observed, when the increase of the chloride and other concentrations were followed. Therefore, there has been a real delay in the dilution of the alcohol solution with the gastric secrete. There was, on an average, a proportional delay in the increase of all the determined constituents of the gastric juice, both at sea level and at 5000 m. The levels reached, however, are different as compared to the pre-experimental average values (Table III) as well as to the values obtained in the carbaminoylcholine tests. The pH values are lower than the corresponding

pre-experimental or carbaminoylcholine figures. The titratable acidity, on the other hand, does not much exceed the average values of the carbaminoylcholine experiments. This, of course, points out to the presence of less buffering substances in the alcohol experiments. The calcium values are on the low side in the alcohol series. Pepsin, again, shows approximately the same behaviour as the titratable acidity. The juice obtained on an alcohol stimulus is, therefore, relatively poor of Ca and of buffers, whether collected at sea level or at 5000 m.

The chloride values of all the subjects were proportional to the values of the titratable acidity. After alcohol, there was no difference between the subject A and the subjects E and O; when the chloride values were plotted against the titratable acidity, all the values were distributed approximately along a common axis. The difference between the regression coefficients was very small and statistically insignificant. Therefore, the non-chloride components in the gastric contents of A were relatively prevalent only in the pre-experimental secrete and after carbaminoylcholine stimulation, whereas after alcohol the secrete had a chloride concentration proportional to the acid secretion in the same way as in the other subjects.

C. EXPERIMENTS ON THE ACHLORHYDRIC SUBJECT

Four tests were performed on the achlorhydric subject Y. Two of these were alcohol tests at sea level, one an alcohol test at 5000 m, and the fourth a carbaminoylcholine test at 5000 m. The stomach tube was felt very irritating and often gave rise to small haemorrhages; therefore, the tests were discontinued.

In the alcohol tests there was a great initial drop in the concentration of the dye, about 50 per cent of the intensity of the colour. After this, the speed of the further dilution did not markedly differ from that observed in the other subjects. The initial drop must probably be attributed to the intense adsorption by the neutral mucosa.

The pH of the samples varied between 6.80 and 8.25. No distinct tendencies could be observed; there were also no differences between the tests at the different altitudes. The titratable acidity varied between 0 and 7.

The chloride values in the pre-experimental samples and in the carbaminoylcholine tests varied between 48 and 81 milliequivalents per litre. In the alcohol experiments a chloride level of more than 70 millieq. was reached before the end of the test. Again, there was no difference between the two altitudes.

The Ca concentration of the pre-experimental and carbaminoylcholine samples varied between 4.3 and 5.4 mg per cent. In the alcohol experiments, the maximum Ca value observed was only 2.6 mg per cent.

There was probably no secretion of pepsin. The peptic activity varied between 0.01 and 0.16 units per ml.

On the whole, there was in these few experiments no evidence indicating any effect of anoxia on the secretory activity of the achlorhydric stomach.

DISCUSSION

The secretion of any gland can be viewed from two standpoints; first, as a purely physico-chemical process, in which one or more of the components of the medium are actively kept in different concentrations on the two sides of the secreting membrane, while the other components are distributed simply according to the laws of diffusion and of Donnan equilibrium. Another standpoint is to regard a secrete as a system — solution — of a more or less fixed composition of which so and so many millilitres are produced per minute.

When the present work was planned, both these standpoints were considered. Anoxia was known to decrease the secretory response of the stomach towards standard stimuli. It could be expected that anoxia might change the proportions of the different components of the gastric juice so as to reduce any osmotic gradients between the gastric contents and blood; on the other hand, it might simply reduce the amount secreted by the different glands.

The results obtained show, that there was a reduction in the secretion of chloride more or less parallelly with the decrease in the acidity of the gastric contents; and this occurred whether the chloride concentration was above or below the blood level. It is, therefore, more appropriate to treat the present results rather in the terms of a gastric secrete being poured into the stomach by the various glands of its walls than in the terms of a physico-chemical equilibrium between blood and the secrete.

The secretion of all the components analysed was decreased in anoxia in both series of experiments, both after alcohol and after carbaminoylcholine, but — curiously enough — the suppression was much less severe in the alcohol experiments. One tentative explanation to this discrepancy can be offered. The stimulation

with alcohol is indirect, whereas that by vagomimetic drugs affects the secretory mechanism at the same point as the normal vagal stimulation. The stimulus in the first case is the presence of irritating fluid in contact with the gastric mucosa. It may be expected that the stimulus persists as long as the contents are not made 'neutral' from the point of view of the chemosensitive elements of the gastric mucosa. (Some adaptation of them, of course, might occur — to what extent, can not be stated). If the secretory response is less than usually, the stimulus will consequently persist for a longer time, and thus in some degree compensate for the decreased secretory response. When, however, a vagomimetic drug is used as a stimulus, this possibility of compensation does not come into question, and a more pronounced effect of anoxia is to be expected. Whether there are other factors contributing to the observed difference, is not known.

SUMMARY

1. The effect of the simulated altitude of 5000 m on the gastric secretions of three normal and of one achlorhydric subjects was investigated. Alcohol and carbaminoylcholine chloride were used as stimuli.

2. In the carbaminoylcholine tests, the volume collected at 5000 m was on an average 1/5, the acid secreted 1/9, and the amount of pepsin 1/12 of the corresponding values at sea level.

3. The total chloride values followed a similar course as the values for titratable acidity, both at sea level and at 5000 m.

4. At sea level there was a negative correlation of -0.44 ± 0.12 between the calcium and titratable acidity values; at 5000 m the calcium values were irregularly scattered.

5. In the alcohol tests the secretion of the different components was fairly uniformly decreased. The suppression of the secretion was less pronounced than in the carbaminoylcholine tests. The cause of this discrepancy between the different stimuli is discussed.

6. The secretion of the achlorhydric stomach seemed unaffected by anoxia in the degree applied.

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